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THE MANIFOLD EFFECT OF SELECTION

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and
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PART I

1. VARIABILITY AND SELECTION

SELECTION, whether natural or artificial, distinguishes in its action only between different phenotypes. Its effects, however, can be permanent, in the sense of persisting after the generation which was subjected to the selection, only to the extent that different genotypes have been differentially favoured. The degree of correspondence between genotype and phenotype is therefore fundamental in determining the permanent response of a population to any selective pressure which it may experience. Genetic differences whose effects are completely expressed in the phenotype will be subject to the full rigour of selection, and will give the maximum immediate response. Genetic differences which are latent, in the sense that their full potential effect is not expressed in the phenotype, will be to that extent protected from selection, and the immediate response to selective pressure will be correspondingly diminished. Selection can in fact operate on this potential genetic variability only as it is released to express itself as free variation in the phenotype.

Thus where selection is of a given constant rigour, the nature of the response to it enables us to discover how the variability was distributed between the free and potential states in the initial population or group. Potential variability which fails to be released will, of course, remain undetected. The limit of advance under continued selection will be determined by the total amount of variability initially available, whether in the free state or in the potential state but capable of being freed. The rate of advance towards this limit will depend on the relative proportions of free and potential variability, and also on the rate at which the latter becomes freed. The greater the proportion of free variability, and the faster the potential is set free, the sooner will the limit of response be reached. The greater the available store of potential variability, and the slower its release, the longer will the advance under selection continue.

Even in the simplest of genetical situations, where the variation of phenotype upon which selection is acting depends on but one gene difference, the variability will be entirely free only where the mating system in force has led to every individual being homozygous for one or other allelomorph. Each allelomorph will then be exerting its full effect in the phenotype, and the variation associated with the gene will be at its greatest. Should there occur any cross-breeding, however, a proportion of the individuals will be heterozygous for the gene. Such a heterozygote may bear a close phenotypic resemblance to one or other of the two homozygotes, or it may lie between them, according to the dominance relations of the gene ; but it cannot closely resemble both homozygotes at once. Thus the effect of one allelomorph must be wholly, or the effect of each allelomorph partially, masked by association in the zygote with its alternative. The variability which is freely expressed as a phenotypic difference between homozygotes is hidden in the heterozygote. Such heterozygotic potential variability, the only type of potential variability possible with a single gene, must always be partly freed by segregation in the next generation, just as it is always produced by the fusion of two unlike gametes in the generation before.

Where the variability depends on two or more gene differences affecting the same character, a second type of potential variability will occur. The effect of one gene may be masked not only by its allelomorph but also by the balancing action of an allelomorph of a different gene. This additional means of storing variability must play an important part wherever there occurs continuous variation mediated by a polygenic system, whose member genes have effects similar to and supplementing one another, and individually small as compared with the non-heritable, or with the total, variation. With two such genes the extreme combinations will be *AABB* and *aabb*, where the particular allelomorphs which are associated have reinforcing effects. In the homozygotes *AAbb* and *aaBB* the genes will then have counter-balancing effects and the difference between phenotypes will

not fully represent the difference in genotype, in the sense that the genes in question can be reassociated to give phenotypic differences of greater magnitude. Potential variability is thus present in the difference between *AAbb* and *aaBB*, but it is not the potential variability of heterozygotes. It is what has been called homozygotic potential variability, and it bears the same relation to heterozygotic potential as does free variability, for it is converted into heterozygotic potential by crossing and released from it by segregation. Thus the homozygotic potential variability must be released more slowly than heterozygotic potential, because it must go through this latter state as part of the process. A minimum of two generations is necessary as compared with a minimum of one (Mather, 1943).

The homozygotic potential state of variability is an alternative to the free state in that when variability passes from the heterozygotic state some of it is freed and the rest becomes homozygotic potential. Where the effective genes are segregating independently the distribution between these two states will be unaffected by the origin of the heterozygote. The actual proportion which passes into the homozygotic potential state, relative to that which becomes free, will of course increase as the number of genes increases: in fact with n genes of strictly additive and equal actions the proportion becoming free is 1/n of the variability which passes from the heterozygotic state. Where the genes are linked, however, the relative proportions taking the two courses are no longer independent of the origin of the heterozygote. If this arose by the fusion of gametes carrying the extreme combinations of genes ($AB \times ab$), a larger part of the variability passing out of the heterozygotic state will become free once more. Equally, of course, where balanced combinations went into the heterozygote ($Ab \times aB$) more of the variability which leaves the heterozygotic state will become homozygotic potential than would be the case in the absence of linkage.

When, as must generally be the case, there is an optimum degree of expression of a character in relation to the selective forces at work, balanced linked combinations of the genes offecting the character will be favoured at the expense of the less balanced (Mather, 1941, 1943). For any pair of additive genes these balanced combinations (*Ab* and *aB*) will exceed the unbalanced (*AB* and *ab*) by an amount depending on the ratio s/c where s is the selective disadvantage of the heterozygous unbalanced types (*Ab/AB*, *Ab/ab*, etc.) relative to the fully balanced types (*Ab/ab*, *aB/aB*, etc.), and c is the recombination frequency of the two genes (Wright, 1944). When $s = c$ the gametes balanced in respect of the two genes will be nearly $2\frac{1}{2}$ times as frequent as the unbalanced, with a corresponding excess of homozygotic potential variability over free. With $s = 2c$ the balanced types will be over 4 times as common as the unbalanced. Thus any tightening of the linkage will lower the proportion of free variability.

This reduction of free variability is, of course, itself the selective

advantage of the linkage. Now the loss of fitness due to the segregation of the unfavourable extreme phenotypes, so far as these are produced by any two genes, is proportional to something between the first and second powers of c, their recombination frequency. Two consequences then follow. On the one hand, any agency making for closer linkage must be favoured, and, on the other, the main balancing effect must be between closely linked genes. In fact the closer the linkage of the genes, the greater becomes their importance in storing variability by balancing one another's action when in combination. Such stored variability can be freed only by recombination, itself rare, in the appropriate heterozygote.

If only one such balanced linkage is broken the release of variability will be a sudden event ; but where, as might be expected with most polygenic systems, balancing is widespread, a number of different recombinations must be recovered, simultaneously or more likely successively, before the variability is freed. The release of variability, and with it advance under selection, will then be a slow, though not always steady, process as the unbalanced, or coupling, combinations are gradually built up from their balanced, or repulsion, forerunners. But once obtained, these coupling combinations will be as easy to keep in being as they were difficult to make, because they can be lost only by the same process of recombination. Response to selection will be as fast wherever it depends on these extreme combinations, as it was slow when the balanced ones were segregating.

The crossing-over by which the balanced combinations are broken down and the variability released, will recombine not merely the polygenic system affecting the character for which selection is practised, but also any other such system, affecting a further character, which might be distributed along the same chromosome. Thus selection for one character will, by picking out the unbalanced combinations of the one set of genes as they are produced by recombination, also pick out unbalanced combinations of other sets of genes mingled with them along the chromosome, and so cause, as a correlated response, change in characters for which no selection is practised. This correlated response could even cause change against selection for the character in question, provided that this selection were of a lower intensity than the transferred effect of that practised on the primary character (Wigan and Mather, 1942).

It is clear from these considerations that the nature of the response to selection will be determined to a great extent by the linkage relations of the polygenic systems concerned. Conversely, of course, suitable observations on behaviour under selection will enable us to infer these linkage relations. The effects of linkage will be expected in all higher organisms, but they should be in strongest evidence where the number of chromosomes is small and the average frequency of crossing-over within them is low. *Drosophila melanogaster* offers, therefore, particularly suitable material for the observation and

analysis of the ways in which advance under selection reflects linkage relations.

The delayed response to selection, due to the necessity for crossing-over to effect particular recombinations, has been seen in experiments on this fly by Mather (1941, 1942) and by Sismanidis (1942). In both cases it was possible to trace the chromosomes in which the recombination had occurred, and Sismanidis also showed that the effect of selection had all the determinacy to be expected from a dependence on linkage relations. He found that the same responses, due to change in the same chromosomes, and depending one must presume on recombinations of the same genes, occurred in lines of the same original constitution, even where these were maintained and selected quite independently of one another. The presence of the expected coupling linkages, which selection caused to replace the former balanced repulsion linkages, could also be inferred from the greater variance of the F_2 between Mather's (1941) high and low selected lines, as compared with that of the F_2 from which they were selected. No selection was, however, made from this second F_2 to show that the limits of selection were as easy to recover as they had initially been difficult to produce.

Correlated responses to selection have been described in *Drosophila* by Wigan and Mather (1942) and in *Lebistes* by Svärdsson (1944). In each case fertility fell, as a concommitant of selection for both higher and lower number of hairs in the flies and for number of fin-rays in the fish. In neither case, however, could the possibility be fully excluded that the relation between the primary character and fertility was simply physiological, due to the same genes affecting both characters, though Wigan and Mather's observations made this possibility the less likely one. A further limitation of these experiments requires notice. Neither of them shows both aspects of the correlated response relation, in the flies the effect of selection for fertility on hair number as well as the effect of selection for hair number on fertility.

Thus while these various experiments have given valuable evidence of the control of advance under selection by the linkage organisation, their results reflect their limitations in scope and time. The experiment with *Drosophila melanogaster*, which we are now to describe, is much more extensive in the number of lines kept, in the time over which the selection has been practised, and in the tests to which the lines have been subjected. Its results, by extending as well as confirming those of the earlier experiments, enable us to see the relation between linkage and selection with increased clarity, and to infer the system of variability with increased confidence.

2. TECHNICAL INFORMATION

The experiment was started on 4th December 1942 by Mr G. M. L. Haskell, who carried it to the 13th selected generation. Circumstances then forced him to give up the investigation, which was continued

thereafter by us. We are indebted to Mr Haskell for allowing us to use his data from the early generations in this publication.

The character chosen to supply the basis of selection was that used earlier by Mather (1941, 1942), *viz.* the number of chaetæ on the ventral surfaces of the 4th and 5th abdominal segments. Though counted separately, the numbers of chaetæ borne on the two segments have in every case been summed to give a single value for the fly. Twenty males and 20 females were counted wherever possible from each culture. Males have on the average some 5 or 6 chaetæ fewer than females (see tables 2 and 3), and so the mean of the two sex means has been used as the measure of the chaeta-producing capacity of the culture. Where, as in the great majority of cases, the full number of individuals could be counted, the mean of the sex means is also the simple mean of all 40 flies ; but where the numbers of the two sexes differed this relation no longer holds. It is then essential to take the mean of the means, rather than the simple mean, in order to avoid distortion by the preponderance of counts from one or other sex.

The start of the experiment was a cross between the two wild-type stocks, Oregon (Or) and Samarkand (Sk). The former has been maintained in this laboratory for over nine years by brother-sister mating, apart from one generation's lapse into cousin mating in the early years. At the time of the cross in question, brother-sister mating had been practised for 74 generations since the lapse, which was itself preceded by 79 known generations of continuous brother-sister mating. The Or line has now reached over 200 generations of unbroken brother-sister mating since the lapse.

The Samarkand stock was being maintained solely by mass culture at the time of the initial cross, but since then an inbred line has been developed by brother-sister mating from it. This has been used side by side with the original mass stock, which is still maintained, for certain tests made later in the experiment. The inbred line and the mass stock gave no evidence in these tests of any difference which might affect the interpretation of the data. In fact the only noticeable difference between them was that flies in the inbred line mated rather less readily than those from the mass culture, and were perhaps less prolific.

An F_1 was raised from the cross between the two wild-type lines, and from it an F_2. Selection was first practised at this stage by the choice of parents for the third generation, which thus became the first selected generation (S–1) of the experiment. Before leaving the F_1 and F_2 generations, it may be noted that the cross was remade and these generations repeated in 1946, for the purpose of observations on the spermathecæ of the females as will be reported in a later section. At the same time Dr M. Westergaard, then visiting this Institution, kindly examined the salivary chromosomes of both F_1 and F_2 without finding evidence of any gross structural difference between the parental lines.

Selection was practised for increasing and decreasing numbers of chaetæ in two separate lines derived from the F_2. In each of these selection lines, and indeed in all the various selection lines of the experiment, two cultures were set up in each generation. As already noted the chaeta numbers were determined for twenty flies of each sex from each culture, and the two most extreme (high in the high selection lines, and low in the low selection lines) were then selected from each twenty flies. The two males from the first of the two parallel cultures of a line were mated jointly to the two females from the second and *vice versa*, so giving two cultures, each with four parental flies, for the next generation. This method of mating was adopted as a means of minimising the risk of inbreeding. Where one of the two cultures of a selection line failed, twenty flies of each sex were taken for counting from the surviving culture in the usual way; but the four extreme flies of each sex were taken and mated two by two, to give parents for the two cultures of the next generation. Thus the failure of a culture meant a halving in the rigour of selection. Failure was, however, relatively uncommon during the experiment.

The four parents of a culture were mated in a vial for from one to three days, according to the circumstances of the moment. They were then transferred to a half-pint milk bottle and allowed to remain in it until just before the first of their offspring began to emerge. The cultures of all lines were placed in the incubator at 25° C. as soon as the parent flies were transferred to them from the vials, and remained at this temperature until finally discarded. With fully fertile flies this technique would ensure crowding in the bottles, but in some of the lines fertility, which was measured throughout the experiment after S–13 by the total output of flies from the bottle, was so low as to prevent the development of crowded conditions. The flies were shaken out of the bottles night and morning, and the sexes stored separately to preserve the virginity of females taken for mating.

As will be seen from later sections, a number of lines were developed and maintained during the experiment by mass mating. Only one culture was made of each of these lines per generation. The mean chaeta number was determined from counts of twenty flies of each sex as in the selection lines. The first ten flies of each sex to be counted were then taken, irrespective of their chaeta numbers, to be the parents of the culture in the next generation. Furthermore, these mass cultured lines were kept in step with the selection lines, so that, with the exception of two lines, *viz.* 2 and 8, which will be mentioned later, the representatives of all lines described as occurring in any particular generation of the experiment were in fact raised simultaneously in the incubator.

The flies were collected from each culture, whether of selection or mass line over at least the major part of the emergence period before the twenty of each sex were taken at random from among them for counting. The total emergence was used in estimating

fertility and for the observations on spermathecæ which will be described later. Earlier experience had suggested that time of emergence and condition of food had only a small effect on the average number of chaetæ, and certainly would not seriously distort observations on this character. Special tests were nevertheless made of their effects.

The test of the effect of culture conditions was made using 6 cultures each with 10 male and 10 female parents taken at random from a single culture of the mass line 3 (see section 3*b*). The flies were mated in vials for a day before transference to the culture bottles, 3 of which were two days old and 3 a week old. The two-day bottles were in good condition, but the others were stale, with dark yellow yeast growths and also some mould. A less vigorous stock would doubtless have died in them. The mean numbers (means of sex means) of chaetæ per fly in the offspring obtained from these bottles are given in table 1. The means from the fresh culture bottles are

TABLE 1

The effect of initial culture condition on chaeta number

Condition of culture bottle	Mean chaeta number in culture			Grand mean
	A	B	C	
Fresh—2 days old . .	40·98	40·88	41·25	41·03
Stale—7 days old . .	42·38	42·10	41·53	42·00

all lower than those from the stale ones. The difference between them is significant, between the 0·05 and 0·02 probability levels, but it is just less than one chaeta. The initial state of the culture bottle cannot therefore have a seriously misleading effect on the interpretation of differences so large as those to be recorded in this experiment, particularly as it was not the practise to use culture bottles which had been standing for as much as a week. With rare exceptions, in fact, the flies were put into the bottles two or three days after these had been made up.

A second test concerned the effect of the time of emergence on chaeta number. Two single pair matings were made in the Or stock and cultured in the usual way. The emerging flies were shaken out each morning and evening over a period of ten days. The daily averages of the two cultures taken together are shown for males and females separately and also jointly in table 2. It appears that there is a fall, albeit an erratic fall, in mean chaeta number with time of emergence, and it seems to be more rapid in the early part of the emergence period than it is later. Indeed the grand average for days 8-10 is slightly higher than that for the period 5-7. The fall is, however,

nowhere great. The most extreme daily averages recorded for males are 40·67 and 39·00, and for females 46·94 and 45·32, a difference of less than 2 chaetæ in each case. As would be expected, the extreme values for the mean of the sexes, 43·76 and 42·37, differ by even less. Since the change in chaeta number with time of emergence should be over-estimated by the differences between the extreme values, the

TABLE 2

The effect of time of emergence on chaeta number

	Mean chaeta number on emergence day									
	1	2	3	4	5	6	7	8	9	10
Males . .	...	40·57	40·67	40·11	40·30	39·00	39·42	40·58	39·16	39·54
Females .	46·83	46·94	46·23	45·88	45·61	45·85	45·32	45·58	45·78	46·12
Mean of sexes	...	43·76	43·45	43·00	42·96	42·43	42·37	43·08	42·47	42·83
			43·40			42·58			42·78	

average fall in chaeta number over the main period of emergence seems unlikely to be much over one chaeta per fly. Like the similar difference caused by culture conditions in the previous test, this fall can cause little trouble in interpreting the results of selection. Even the slight effect it could have will have been minimised by the technique of collecting the flies over a large part of the emergence period before taking a sample for counting.

A further possible source of error remains to be mentioned, *viz.* personal differences in chaeta counting. It will be recalled that Mr Haskell made the counts until generations S–13, after which with a few exceptions they were made throughout the experiment by the junior author. On the exceptional occasions the senior author counted the chaetæ. Table 3 shows the average counts made by all three

TABLE 3

Personal differences in counting chaetæ in the Oregon inbred stock

Counter	Time of count	Mean number of chaetæ		
		Males	Females	Mean of sexes
K. Mather . .	1939-40	39·6	44·4	42·0
G. M. L. Haskell .	December 1942	32·0	40·1	36·0
B. J. Harrison .	February 1944	40·9	47·0	43·9
,, ,, .	December 1946	40·8	45·5	43·2

people on the Or inbred stock. The two Harrison counts agree well with each other though separated by nearly three years. The Mather

count of some five years earlier differed by only 1½ chaetæ from the average of the two Harrison counts. Such a difference could be due to a small change in the genetic constitution of the Or line; but it might also have been caused by the change in composition of the culture medium necessitated during the period 1939-46. It is in any case a difference of little moment, and in fact the occasional counts made by the senior author during the experiment agreed well with those of the junior author in all lines.

The situation is somewhat different when we turn to the Haskell counts. These average 6 chaetæ less than the Mather counts and 7·5 less than the Harrison average. The chaeta number of an inbred stock seems hardly likely from genetic causes to have fallen so much only to rise again. The discrepancy therefore suggests a genuine personal difference in counting, and warns us to be cautious in the detailed interpretation of the relations between the first thirteen generations and the rest of the experiment. Attention will later be drawn to certain specific effects of this change in counting.

3. CHAETA NUMBER AND FERTILITY

(a) The low selection and low mass lines

When selection was started in the F_2 of the cross between the Or and Sk stocks it was practised for both low and high chaeta numbers in separate lines. Much the greater part of the experiment is concerned with the high line and its derivatives, but the low line also has its interest. It will therefore be described before attention is turned to the results of selection for high chaeta number.

The progress in chaeta number of the low line under selection is shown for the first 61 generations in fig. 1, and for the next 61 in fig. 2. In these figures, as indeed in all the figures, solid lines are used to portray the chaeta numbers of lines upon which selection was being practised, and broken lines for the mass cultures kept without any artificial selection. Each point represents the mean of the chaeta numbers (each of which is, as already noted, itself the mean of the two sex means) of the two cultures in each generation of the selection lines, except where one of the two cultures failed, in which case the point on the graph is the chaeta number of the single surviving culture. In the mass lines each point is obtained from only one culture, as only one culture was kept in each generation. In referring to these graphs the generation numbers shown along the abscissa will be used, prefixed by an S to indicate that they start from the first generations of selection, *i.e.* F_3 and thus $F_2 = S\text{–}0$, and $F_3 = S\text{–}1$.

Progress under selection in the low line was fairly steadily downwards at first but the chaeta number receded sharply upwards in generations S–9 and S–10, only to fall again until S–15. At this

point the number had fallen by over 5 chaetæ from the F_2 mean value. Then followed a steady recession, amounting to some 2 chaetæ over the next 6 generations. This recession was most likely due to the change in counting consequent on the change in observers at S–13. Probably the second observer, B. J. Harrison, was influenced at first by his predecessor's counts and only gradually over several generations established his own level of counting. After S–22 the advance under selection was resumed and gradually increased in speed until by S–34 the lowest level was reached at 25 chaetæ, an advance of 11 (or quite likely really more in view of the change in counting) from the F_2 mean of 36. Sterility had also set in and only a few males were obtained in S–35 so that the line died out.

The low line thus behaved in the way expected from earlier experience. It progressed slowly, attaining its full advance only after 34 generations. This must indicate that the main progress depended on the release of variability by unbalance following recombination between balanced combinations within the chromosomes; for with only three major chromosomes progress due to the segregation and recombination of whole chromosomes should certainly have been over by S–5. At this point the advance had, in fact, only covered 3 chaetæ and there was a period of stability and even of recession which might well mark the period of waiting before intra-chromosomal recombination effectively began to release the variability upon which the main advance depended. This main advance was accompanied by the correlated response of loss of fertility, which eventually killed the line.

At S–20 a mass line (designated the low mass line or line 1) was started from the low selection line in parallel with one begun at that generation from the high selection line. The chaeta number of this mass line fluctuated somewhat during its history, to an extent perhaps a little greater than could fairly be ascribed to non-heritable effects. Such fluctuations would not be surprising in a mass line where selection is not acting to steady the random variation of gene frequencies. That no more deep seated cause of change was at work is attested by the remarkable overall stability of this mass line during the 95 generations that it was in existence. The line began with a mean of just over 32 chaetæ in S–21, and it had a mean of about 33 when it was terminated in S–115. Its peak value was 35, reached at S–44 and again at S–95, and its lowest value 30½ at S–27, with a value of 31 attained or just passed six times.

A new low selection was taken off line 1 at S–31 and progressed to a value of 29 chaetæ in the 8 generations which elapsed before sterility killed it at S–39. A third low line begun at S–42 eventually died out after only 3 generations, during which progress had been of only 1 chaeta. The fourth low selection started from the mass at S–48, survived one generation longer, and progressed for nearly 3 chaetæ before it was extinguished by its sterility at S–52. The

next two low selections, at S–53 and S–54, were sterile from the first, inasmuch as no progeny were obtained from the parents originally selected from the mass line. The last low selection, attempted at S–56, survived one generation before sterility overtook it.

Selection from line 1 in the opposite direction, for high chaeta number, fared little better. Two such selections were attempted. One, begun at S–48, terminated through sterility after only two generations. Its chaeta number showed no advance; indeed it approximated so closely to that of the mass line that clarity demanded the omission of this selection line from fig. 1. The second high

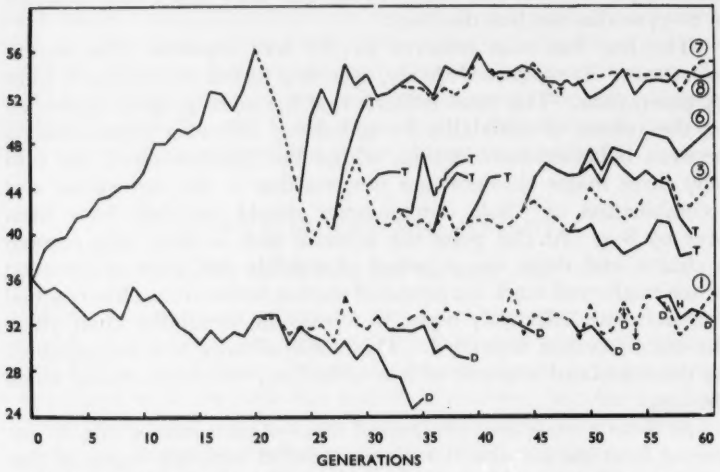

FIG. 1.—Response to selection between S-0 and S-61 for abdominal chaetæ in lines 1, 3, 6, 7 and 8 and certain other lines which were not continued. Selection lines are solid and mass lines broken. T indicates that the line was terminated deliberately, and D that it died out through sterility. The selection marked H was a high selection from line 1, the other selections from which were for low chaeta number. Note the low selections from lines 3 and 6 at S-48 and S-49 respectively. Ordinate is mean number of chaetæ.

selection was a little more successful. Begun at S–56, it survived until S–61 before it was extinguished by sterility. It advanced little if at all, from the parent line in chaeta number, as can be seen from fig. 1 where it is distinguished from the various low selections, taken off the same mass, by the letter H.

Thus selection from the low mass line became progressively more difficult as the generations wore on, in the sense that both length of survival and progress made became lower. Some progress was made, however, in the second selection, and a little even in the fourth, before sterility supervened, so that the stability of the low mass itself can hardly have been due to homozygosity. Rather it seems that the low mass was building up a balanced sterility system within itself

and in the absence of any artificial intervention. The flies with the more extreme chaeta numbers seem to have been effectively sterile when the system was eventually fully developed—a sterility which might have been due either to their being more homozygous than their less extreme fellows, or to the combinations for which they were heterozygous being less well relationally balanced. The short periods of survival of some of the later selection lines could well have been due to flies with better balanced genotypes being selected among the 8 parents, with which each line commenced, owing to their extreme chaeta numbers being lower than usual in consequence of non-heritable effects. Such flies would keep the line going, though with little or no advance under selection, until among their descendants only genetically extreme, and hence sterile, flies were selected as the parents of a generation. Then the line would die out. The behaviour of all the selection lines after S–40 would accord in this way with the existence of a balanced sterility system in the mass line, a system which the second low selection suggests to be partially, though of course not fully, developed as early as S–31.

We shall meet several other cases of balanced, or partially balanced, sterility during the course of this report; but that in the low mass line was remarkable among them in two respects. First, it was of sharper action, in that deliberate selection from the mass became virtually impossible; and second, it seems to have grown in strength during the history of the mass line itself.

Three attempts were made to obtain more direct evidence of the sterility in this low mass line. In generation S–75, 27 flies of each sex were counted for chaeta number and then made up into 27 pair matings. For this purpose those of each sex were divided into three groups, each of 9 individuals, with the highest (H), the middle (M) and the lowest (L) chaeta numbers respectively. They were then mated to give 3 H×H matings, 3 H×M and so on, as shown in table 4 where the actual chaeta numbers are given. The mated pairs were allowed to breed, each in a single vial, and the fertility or sterility was recorded according to whether offspring were produced or not. The table shows that all 9 females in the M class gave offspring, while only 5 from each of the H and L classes did so. The M males had one more fertile mating than the H or L males. There is thus an indication, though hardly significant evidence, that females with medium chaeta numbers were more often fertile than their extreme fellows. In the case of the males the differences between the three classes were too small to be regarded as even suggestive. The 18 fertile matings averaged 38 progeny each.

The second test was made by mating 15 females and 20 males individually to single mates from the Or line, after counting their chaetæ (table 5). Fertility of the line 1 females was judged by the presence of larvæ in the culture vials, and of the males by the presence of live sperm in the receptacles of their mates after two days. There

is a suggestion that the females with low chaeta numbers are more likely to be sterile than those with higher ones, but the difference in

TABLE 4

Chaeta number and fertility of matings in the low mass line (S-75)

Females \ Males	H	M	L	
H	36×36 36×35 38×33 1f+2s	39×32 41×33 36×31 2f+1s	37×30 36×30 37×27 2f+1s	5f+4s
M	35×35 35×34 34×33 3f+0s	35×32 35×32 35×32 3f+0s	35×29 35×26 35×29 3f+0s	9f+0s
L	34×33 31×35 33×33 2f+1s	32×32 34×31 34×31 2f+1s	34×30 32×30 33×30 1f+2s	5f+4s
	6f+3s	7f+2s	6f+3s	

The numbers are the chaeta numbers of the parent flies
f = fertile and s = sterile culture

TABLE 5

Fertility of single flies of low mass line in crosses to Oregon

Females—Fertility judged by presence of larvæ after mating with Oregon male

Chaeta number	f 33	s	f 34	s	f 35	s	f 36	s	f 37	s	f 38	s	f 39	s
Number of flies	1	1	...	2	4	...	2	1	3	...	...	...	1	...

Mean chaeta number of
Sterile females 34·25
Fertile females 35·91
} Difference not significant

Males.—Fertility judged by presence of live sperm in Oregon female

Chaeta number	f 28	s	f 29	s	f 30	s	f 31	s	f 32	s	f 33	s	f 34	s	f 35	s	f 36	s
Number of flies	...	1	...	3	2	1	2	...	4	1	3	...	2	...	...	...	1	...

Mean chaeta number of
Sterile males 29·50
Fertile males 32·36
} Difference significant (P 0·01-0·001)

mean chaeta number of the sterile and fertile females is not significant. It might, however, be noted that, as in the first test, no female with 35 chaetæ proved sterile. The test of males proved more conclusive.

Of these 6 were sterile, including 5 of the 6 flies with the lowest chaeta numbers. The mean chaeta number of sterile males was 2·86 below the average of their fertile counterparts, a difference which is significant with a probability only just above 0·001.

The third test was made on four pair matings in S–57, both males and females coming from line 1. The paired flies were transferred to new vials daily, to permit counts to be made of the eggs laid (table 6). The average daily output of the 4 females was only 18·4

TABLE 6

Fertility of eggs from four matings in low mass line (S-57)

Mating	Eggs laid	Progeny obtained
37×35	68	0
33×27	19	0
32×35	156	54
31×26	125	0

Chaeta number of female parent given first

eggs, with 63 as the most laid by any one fly on any one day. Progeny were obtained from only one mating and then to the extent of only about $\frac{1}{3}$ of the eggs laid. This fertile pair consisted of a female with a moderately low and a male with a moderately high chaeta number.

The evidence from these tests can hardly be regarded as conclusive. They do show, however, that the flies of line 1 had a fairly high incidence of sterility, and that the output of progeny by even a fertile pair was low. There is also an indication from the first test that the females with more extreme chaeta numbers were less often fertile than those with central counts ; and from the second test that males with low chaeta numbers failed to inseminate their mates more often than those with higher numbers.

So far as they go, therefore, these tests agree with the view that the low mass line had developed a balanced sterility system within itself. Of how it came to be so sharp in its action we have no evidence except that, from the fates of the successive attempts to select from the mass, the rise of system would seem to have been gradual. A further investigation of the system would clearly have demanded very elaborate experiments, more costly in both time and space, and less informative on the questions with which the work was started than those which had become necessary in the high counterpart of the low selection line and its derivatives. The investigation of the line 1 was therefore abandoned and attention concentrated on the high selection and its derivatives, to which we must now turn.

(*b*) *The high selection line and its derivatives*

The high selection line was started at the same time and from the same F_2 as its low counterpart. Its progress in chaeta number

was both more rapid and more smooth than that of the low line, without any suggestion of a prolonged pause after the first few generations (fig. 1). By generation S–5 it had risen from the initial chaeta number of 36 to 41½, by S–10 to 45, and by S–15 to 49½. It would thus appear that recombination within chromosomes must have begun to release variability, so as to permit further response to selection, as soon as, or even before, the response due to the reassortment of whole chromosomes came to an end. The effect of the change in counting after S–13 is not particularly noticeable, perhaps because it served to mask a period of reduced progress. However this may be, the rise in chaeta number continued, though a little erratically in the last 4 generations, up to S–20 when the chaeta number of 56 was attained, representing an increase of 20 over the initial number.

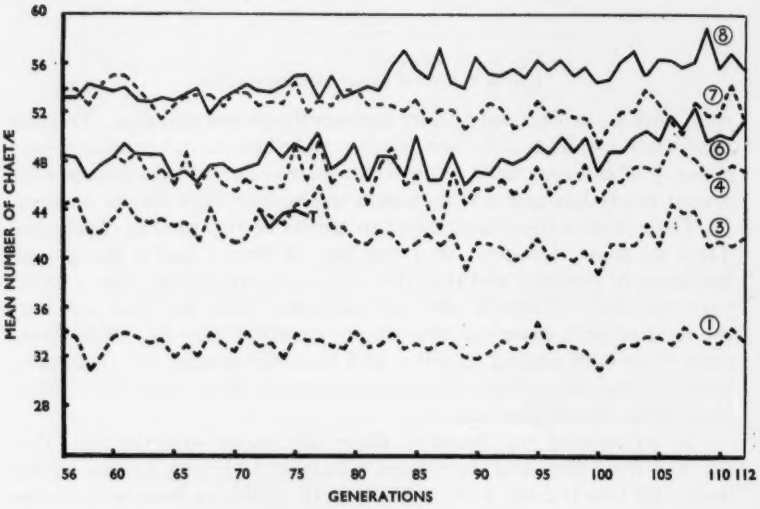

FIG. 2.—As fig. 1, but covering generations S-56 to S-112. Line 4 appears for the first time at S-60 as the mass taken from line 6.

As in the low line, fertility fell in the later stages of the advance in chaeta number. The fall was, as might be expected from the rates of change in chaeta number, more rapid in the high line than in the low, and indeed a total of only 8 flies was obtained from the two cultures of S–20. To continue with selection appeared therefore likely to result only in the total loss of the line in S–21 or soon after, and resort was made to mass culture. In this mass line (line 3), which replaced the high selection line, the average chaeta number immediately began to fall very sharply, and by S–25 was down to little more than 39, a loss in 5 generations of over 80 per cent. of

the gain which had taken 20 generations to achieve. Indeed, in view of the change in counting at S–13 it may well be that the whole of the selective advance had been lost in these 5 generations of mass culture.

The fall in chaeta number checked at S–25 and an erratic rise from a mean of 39 to one of 46 seems to have occurred during the next 4 generations of line 3. It then fell back to 41 by S–31, after which it fluctuated much as the line 1 did. Line 3 suggests, however, a number of longer and slower changes superimposed on the erratic, non-heritable and chance fluctuations which formed the only variation in the line 1. There seems to have been a slow rise towards 44 chaetæ between generations S–42 and S–55, followed by a period of general stability until just after S–70 (fig. 2), when a small rise took place, only to be followed in its turn by a steady decline to a general level of 40 chaetæ at S–100. A further rise then appears to have occurred to S–106, but some doubt is cast on this by the more or less parallel behaviour of the three other mass lines of fig. 2. Possibly the environment, common to all the lines, was changing at this point. In any case there came a fall to S–109, after which the mean chaeta number was stable (leaving aside the customary small fluctuations) till the end of the experiment (fig. 3.).

Even if their occurrence be taken as real, these long, slow and not very large changes in chaeta number are no more than might be expected in what, as we shall presently see, must have been genetically a heterogeneous population, and one whose degree of heterogeneity was itself changing. It is the rapid fall in chaeta number between S–20 and S–25 which must attract our main attention, rather than the near stability which followed.

The fall in fertility of the high selection line is the customary correlated response to selection for changed chaeta number. The recombinant chromosomes picked out by their changed, and, from the standpoint of the original stocks, unbalanced combinations governing chaeta number, are by virtue of the same recombination also unbalanced for the polygenic system governing fertility. And genic unbalance in respect of fertility can only mean one thing—increased sterility.* Thus these recombinant chromosomes, giving the higher chaeta numbers which artificial selection favours, also change fertility in a way which natural selection must disfavour. They are maintained in the line only because the artificial selection is stronger than the natural selection.

Should the effect on fertility not be too disastrous, the selection for chaeta number will eventually fix the chromosomes with the

* In these experiments sterility of the parents is generally inseparable from inviability of the offspring. Both give sterility of the line. The single term sterility will therefore be used to cover both phenomena. The arguments relating to recombination, unbalance and correlated response will obviously apply as much to viability as to true sterility, and so of course also to sterility used in the present sense to cover both phenomena simultaneously.

most changed combinations by rejecting all the ancestral and less changed types in the population. Where the flies contain only the combinations giving reduced fertility, relaxation of selection for chaeta number will merely result in the line maintaining the level that it has attained in chaeta number, for there will be nothing to change it. But if the more changed combinations have not fully ousted the

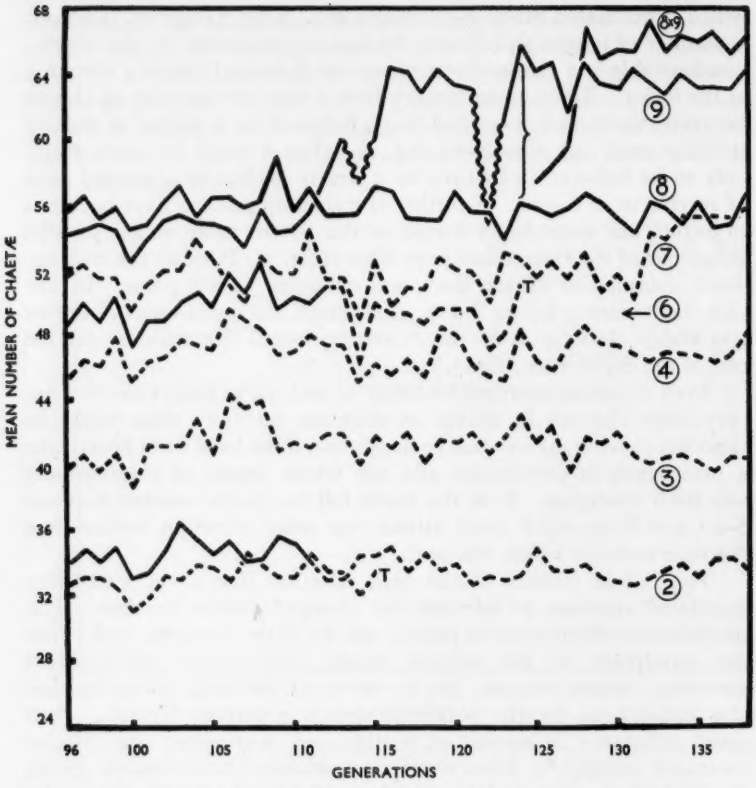

FIG. 3.—As figs. 1 and 2, but covering generations S-96 to S-138. Lines 2 and 9 are also included: their origins and earlier behaviours are shown in figs. 6 and 5 respectively. The wavy lines show where selection had to be relaxed in line 9 owing to infertility.

parental and less changed ones, owing to an overdrastic reduction in fertility when homozygous, this relaxation of the selection for chaeta number will expose the more changed combinations to competition from the less changed. The latter give the greater fertility and so, when the inability to bring about high chaeta number is no longer an agent in determining their fate, they will multiply at the expense of the changed types, and chaeta number must fall.

We can see in fact the reverse side of the correlated response to selection in this situation.

This seems to be exactly what has happened in the high line. So long as artificial selection made chaeta number the capital character and fertility the subordinate, chaeta number rose even though fertility was falling. The line failed, however, to lose all representatives of the chromosomes having parental or at least less changed effects on chaeta number and fertility, doubtless because flies carrying nothing but the most drastically altered combinations were sterile or inviable or nearly so. Thus when artificial selection was relaxed another type of selection took charge. Owing to the natural selection of competition under the conditions of mass culture, fertility became the capital character and chaeta number the subordinate. The less changed combinations multiplied so that fertility rose and chaeta number fell, just as chaeta number had risen and fertility fallen under the artificial selection. Thus the two compound responses to the two types of selection owed their occurrence to the same phenomenon. Intermingling of the two polygenic systems along the chromosomes resulted in recombination causing them to change simultaneously. The high chaeta combinations of necessity came to be associated with lowered fertility, so that no matter which was rising under selection, chaeta number or fertility, the other must fall.

There must, however, be a difference as well as a resemblance in the two aspects of the correlated response as revealed by the changes under the two types of selection. When chaeta number was rising and fertility falling, they were doing so only as recombination released the hidden variability by producing changed polygenic combinations. The advance under selection depended on the building up of new systems of genes, and the speed of advance could only be the speed at which crossing-over could reassociate the effective genes. Several, if not many, cross-overs might be needed to do this within one chromosome. When natural selection for fertility took charge, on the other hand, its discriminative effect would be exercised between chromosomes carrying widely divergent combinations of genes already in existence. The rise in fertility and fall in chaeta number in the mass line had not to wait on recombination by crossing-over, so that the change was much more rapid than its opposite which had occurred under the artificial selection of the high line.

Thus once obtained, the combinations built up slowly through the agency of crossing-over behave virtually as units in inheritance ; they are, and indeed must be, as easy to maintain as they were difficult to make, because they can break down only by the same process that built them up. We have seen that the fall in chaeta number of the line 3 was much faster than the previous rise in the selection line for this reason. Further rises in reselection lines taken off the mass line should, however, be as fast as the fall had been, because they would depend on the utilisation of the high chaeta number combinations

already available, though with diminishing frequency, in the mass line. Reselection should in fact reverse the process whose effects had been seen in the rapid fall of the line 3, rather than repeat the process which had given the slow rise of the first selection.

Eight reselection lines were taken off the line 3 at various times. The first (line 8) was begun at S–24, a generation before line 3 reached its low level, and rose so rapidly that in 4 generations it had reached within about one chaeta of the peak recorded at S–20 in the first high selection. Here it became effectively stable in chaeta number and remained so until S–80 even though selection was continued. Its later behaviour will be described in section 3 (*f*). The important point for our present purpose is that this line recovered the high chaeta number as rapidly as the line 3 had lost it. The high chaeta combinations, which as units had dwindled in frequency under the natural selection of the mass culture, increased as units once again when artificial selection was reapplied. The slow process of building them up by crossing-over had not to be repeated.

One further point remains to be noticed about this reselection. Unlike the first selection line, the high chaeta number was attained without an overdrastic loss of fertility. The number of flies never fell so low as to endanger the stock. Furthermore, when a second mass line (line 7) was made from this reselection at S–34 its mean chaeta number did not fall in the way that that of line 3 had done. The selection must have been effective in making the line homogeneous for high chaeta number combinations, so that relaxation of the selection for chaeta number did not result in its fall. There must have been between S–20 and S–34 a sufficient reassociation of the polygenes governing chaeta number and fertility for the high chaeta number combinations to acquire sufficient fertility to give a stable line. That this line was only homogeneous for high chaeta combinations in the various chromosomes, and not homozygous for any of them, will become clear when we consider its subsequent behaviour in section 3 (*e*).

The second reselection was taken from line 3 at S–27. By S–30 it was so obviously repeating the behaviour of its fellow, begun 3 generations earlier, that it was deliberately terminated. Line 3 then fluctuated upwards in chaeta number for 2 generations before it fell again, and during this fluctuation its genetical constitution must also have changed, for the next 5 reselections, taken off at S–30, S–33, S–36, S–39 and S–44, rose each in 2 generations to a mean chaeta number of 45 or 46 at which level the rapid advance ceased. They had gone up rather less than half-way to the level reached by the reselections of S–24 and S–27. The change in the line 3 between S–27 and S–30 had evidently permanently reduced the variability available for response to selection by rather more than half, just as would happen if the high chaeta number combinations in at least one and at most two of the three major chromosomes had finally

dwindled in frequency so far under the conditions of competition in mass culture that they had vanished altogether. Having been relatively rare before, their total loss had no detectable effect in lowering the mean chaeta number of line 3 : it could only be detected by the loss of half the capacity to respond to reselection.

The last of the 5 reselection lines (line 6) which rose rapidly only to the halfway mark, *viz.* the one begun at S–44, was maintained under selection for some 80 generations. It showed no further rapid advance, though it rose slowly by 4 chaetæ in the 12 generations after S–49. It then became stable until about S–93, when it began another slow rise ending some 14 generations later. A mass culture line (line 4) begun from it at S–60 remained as stable as a mass line could be expected to do until the end of the experiment (figs. 2 and 3). Thus like line 8 at the top level, developed by the first reselection, line 6 must have been homogeneous for high chaeta combinations in such chromosomes as had retained in line 3 the variability upon which response to selection depended ; but it could not have been homozygous for any of them. Its power of rapid advance, which depended on the rise in frequency of existing high chaeta number combinations, was expended in the first 2 generations, but the line could and did advance further as new combinations giving even higher numbers were built up slowly by crossing-over just as in the first 20 generations of the experiment.

The change in line 3, whose effect we have just seen, was not the last to occur. At S–72 the eighth high reselection was begun from this mass by Mr S. H. Revell, to whom we are indebted for permission to make use of his results. He failed to obtain any advance in chaeta number in the 4 generations during which he practised selection (fig. 2). The fate that had overtaken the high chaeta combinations of one, or possibly two, of the three major chromosomes between S–27 and S–30 had extended to those of the remaining chromosomes. None of those combinations producing high chaeta numbers built up by the selection of the first 20 generations of the experiment had survived in the mass line up to S–72. Any further increase in chaeta number under selection must thus depend once again on the slow process of recombination within the chromosomes.

(c) *The chromosome assays*

The responses to selection enable us to see how high chaeta number combinations are built up slowly and piecemeal by crossing-over under selection ; how they are associated with reduced fertility ; how, once in being, they act as units when they are decreasing in frequency by virtue of natural selection for fertility, or increasing in frequency under reselection for high chaeta number ; and how they are even finally lost as units in the mass line. These results do not, however, enable us to specify which particular chromosomes have

been affected by these changes, or how much each chromosome has contributed to the responses to selection at the various stages of the experiment. This information can be gained in a large measure, and the general inferences drawn from the results of selection verified, through the use of the technique of chromosome assay described by Mather (1942).

The method of assay depends on comparisons of the chaeta producing power of the chromosomes from the various lines, developed in the experiment, with their homologues from a common tester stock. The homologous chromosomes of the various lines can then be compared with one another by means of this common standard of reference. Direct comparison of the chromosomes from the various lines is of course impossible because the lines all being wild-type, their chromosomes cannot be followed in segregation. The chromosomes of the tester are, however, marked with dominant major genes so that their segregation from their homologues can be followed. Inversions are used in the tester chromosomes to reduce their recombination in the heterozygotes, so that they segregate sufficiently well as whole units, the marker genes enabling these units to be traced in transmission.

The tester used was a stock of *ClB*/+ ; *Cy* L^4/*Pm* ; *H*/*Sb* *In* 3(*R*)*Mo*. Females from it were crossed with males of the line to be assayed ; and females of the type *ClB*/+ ; *Pm*/+ ; *Sb*/+ were taken from the F_1 and crossed back to males of the experimental line. In this backcross 8 classes of female progeny are to be expected, in all of which one representative of each chromosome will have come through the father from the line under assay. The other representative, that from the mother, will sometimes be derived ultimately from the line (L) and sometimes from the tester (T), the origin being indicated by the presence or absence of the marker gene, as shown in table 7. The same will be true of the autosomes in the males from the backcross, but not of the X. Males have, therefore, not been used in these assays, and the data given in this section refer always to females unless otherwise stated.

The chaetæ were counted on a maximum of 5 females of each of the 8 classes in each of the replicates of the backcross. Usually 8 (though occasionally 10) replicates were used and the class averages thus determined from about 40 flies. Now classes 1 and 5 in table 7 differ only in that 1 has an X chromosome each from the tester and the line, while 5 has both X's from the line. Thus the difference in average chaeta number of these classes affords an estimate of the excess in chaeta producing power of the X from the line over its homologue from the tester. The same is true, however, of classes 2 and 6, 3 and 7, and 4 and 8. So we can obtain a pooled estimate of the excess by finding $\frac{1}{4}$ $(\bar{8}+\bar{7}+\bar{6}+\bar{5}-\bar{4}-\bar{3}-\bar{2}-\bar{1})$ where $\bar{8}$ is the average number of chaetæ carried by females of class 8 and so on.

The data can be made to yield estimates of the excess chaeta producing power of chromosomes II and III from the line over their homologues from the tester by adding and subtracting according to the signs in columns 7 and 8 of the table. Since the experiment is

TABLE 7

The method of chromosome assay

Class	Phenotype	Source of chromosomes			Combinations measuring						
					Main effects			Interactions			
		X	II	III	X	II	III	X-II	X-III	II-III	X-II-III
1	*B Pm Sb*	T, L	T, L	T, L	−	−	−	+	+	+	−
2	*B Pm*	T, L	T, L	2L	−	−	+	+	−	−	+
3	*B Sb*	T, L	2L	T, L	−	+	−	−	+	−	+
4	*B*	T, L	2L	2L	−	+	+	−	−	+	−
5	*Pm Sb*	2L	T, L	T, L	+	−	−	−	−	+	+
6	*Pm*	2L	T, L	2L	+	−	+	−	+	−	−
7	*Sb*	2L	2L	T, L	+	+	−	+	−	−	−
8	wild-type	2L	2L	2L	+	+	+	+	+	+	+

T, L = 1 chromosome each from the tester and the line
2L = both chromosomes from the line

factorial in type these estimates will be independent of one another and of the estimate of the effect of the X (see Fisher, 1947 ; Mather, 1946). The analysis can be completed by finding the so-called interactions in effect of the chromosomes taken two at a time and all three together, using the functions in columns 9-12. These interaction items measure the departure from simple additiveness of the effects of the various chromosomes in their different combinations. All the totals arrived at by using these functions are divided by 4 as in the case of the X. The functions are orthogonal to one another and the values obtained by their use are independent, so that each of the seven (three main effects, three first-order interactions, and one second-order interaction) can be manipulated and interpreted as a separate entity.

Before turning to the consideration of the assay data themselves, two limitations to the technique must be noted. Since the comparison for each chromosome is between the heterozygote T/L and the homozygote L/L, the effects of the genes from the line, L, will escape detection where these genes are completely dominant to their allelomorphs from the tester, T. Only fully recessive genes from L will exert their full effects in this comparison, those of intermediate dominance exerting corresponding fractions of their effects. If dominance is absent or is balanced in the sense that the total dominance of genes from L is equalled by that of the genes from T, the assays

will account for half the genetic difference in chaeta producing power. Or to put it another way, they will be 50 per cent. efficient.

The second limitation is that the inversions used, especially those in chromosomes X and III, are known not to suppress all effective recombination. The comparisons will thus not always be between the homologous chromosomes of T and L as units. Some of the genetic differences will escape detection, or will appear as having a spuriously low effect. The loss of efficiency of the assay due to this cause should not, however, be great because, even though not eliminated, recombination is very materially reduced. This expectation is borne out in practice. The flies of the wild-type and the *Clb*/+ ; *Pm*/+ ; *Sb*/+ classes in the backcross should differ in average chaeta number by as much as the flies of the line itself and of the F_1 between line and tester, apart from loss due to recombination. Table 8

TABLE 8

Loss of efficiency of assays through recombination

The entries in this table are the differences in chaeta number between wild-type flies and *Clb*/+ ; *Pm*/+ ; *Sb*/+ when these are

A = from the line under assay and the F_1
B = segregant classes from the backcross

Line	A	B	Flies for assay taken from
Sk inbred . . .	−9·1	−5·6	...
Sk mass . . .	−8·8	−5·5	...
Line 1 . . .	−6·3	−4·9	S-110
Line 2 . . .	−6·6	−6·0	S-109
Line 3 . . .	−2·1	−1·0	S-108
Line 4 . . .	0·6	3·5	S-109
Line 5 . . .	2·3	3·5	S-109
Line 6 . . .	2·0	3·7	S- 97
Line 7 . . .	5·0	6·0	S-103
Line 8 . . .	8·8	7·0	S-108
Line 9-1 . . .	6·6	6·2	S-107
Line 9-2 . . .	13·4	11·6	S-124
Average neglecting sign	5·97	5·38	...

compares these differences in 12 different assays. Those in the backcross (column B) are generally speaking a little lower than those between line and F_1 (column A) ; but this loss is on the average only 10 per cent. of the column A difference. The loss of efficiency due to recombination, with which of course is confounded the loss due to failure to assay chromosome IV, is thus insufficient to cause any serious misjudgment of the situation. There is a suggestion that the loss is greater in the lines with lower chaeta numbers, particularly the parental Sk line, but the data are not fully consistent in regard to this relation between chaeta number and loss.

The parental Or line was assayed in 11 tests, at least one such being undertaken whenever other lines were being tested. Since the

Or line was inbred and so genetically uniform, or nearly uniform, throughout the experiment, these 11 assays afford a measure of the consistency of the method, or looked at the other way of its error variance. Their results are set out in table 9. The 11 values for

TABLE 9

The eleven assays of the Oregon parent

	Main effects			Interactions				
	X	II	III	X-II	X-III	II-III	X-II-III	
	0·83	0·44	−3·05	0·07	0·46	−0·15	−0·16	
	0·52	0·12	−2·12	1·12	0·58	0·78	0·58	
	1·68	0·46	−2·44	0·21	−0·12	0·79	−0·24	
	1·08	0·26	−0·79	−0·32	−0·57	0·67	0·01	
	1·03	0·97	−3·45	−0·81	−0·21	0·71	−0·70	
	0·40	0·94	−2·90	−0·30	−0·19	1·22	−0·46	
	0·75	0·47	−1·22	−0·04	−0·26	0·47	0·13	
	0·49	0·47	−2·48	0·21	−0·68	1·08	−0·03	
	0·39	0·57	−2·09	−0·93	−0·92	−0·10	1·31	
	0·52	0·47	−3·43	−0·63	0·66	0·78	−0·02	
	0·98	0·73	−2·87	−0·24	−0·23	0·16	0·03	
Average . .	0·79	0·54	−2·44	−0·15	−0·14	0·58	0·04	
Analyses of variance—mean squares								N
Grand deviation .	6·81	3·18	65·50	0·25	0·20	3·75	0·02	1
Heterogeneity .	0·15	0·07	0·72	0·33	0·26	0·20	0·28	10
Pooled heterogeneity	0·2869 for N = 70							

each of the 7 independent comparisons (table 7) are given, together with their averages. The variance of each of the 7 comparisons can be analysed into two parts, one measuring the grand deviation from 0 and based on 1 degree of freedom (N = 1), and the other measuring the heterogeneity of the 11 corresponding estimates and based on 10 degrees of freedom (N = 10). The 7 heterogeneity mean squares are consistent with one another in value except possibly that for the main effect of chromosome III. Even this is significantly greater than the pool of its fellows only at the 1 per cent. level, and when we remember that it was picked out as the largest value of seven, it seems that we should not regard it as inhomogeneous with the rest. The 7 heterogeneity mean squares may therefore be pooled to give a joint error mean square of 0·2869 based on 70 degrees of freedom. Thus single estimates of the various comparisons will have a standard error of $\sqrt{0{\cdot}2869}$ or 0·5356, while averages of two estimates will have a standard error of $\sqrt{\frac{1}{2}\times 0{\cdot}2869}$ or 0·3787, and averages of n estimates a standard error of $\sqrt{\frac{1}{n}\times 0{\cdot}2869}$.

The grand deviations of all three main effects are significant, so showing that Or differs from the tester in all 3 chromosomes. It will be observed from the average comparisons that in both X and II Or has a greater chaeta producing power than the tester, but that the joint effect of these 2 chromosomes, 0·79+0·54, is more than offset by the lower chaeta producing power of chromosome III, *viz.* −2·44. Thus the Or chromosomes have overall a lower chaeta producing power than the particular chromosomes used from the tester, though the differences are partly balanced.

There is no evidence of interaction between the genes in the different chromosomes except between II and III. This interaction is in the positive direction, *i.e.* the combinations found together in the line and the tester have greater effects than would be expected from their individual behaviour taken over the segregating families as a whole.

Twelve other lines were assayed (table 10) six of them in duplicate. Furthermore two of the twelve were effectively duplicates, since one was the Sk mass line from which the parents of the original cross were taken, and the other was the Sk inbred line derived from this mass. Each of these two was done in duplicate, so that in all we have 4 assays of Sk. The homogeneity of the various duplicate assays, and of the quadruplicate assay of Sk, was tested by comparison with the pooled error mean square obtained from the Oregon tests. All that need be said is that with one exception, line 7, no difference could be detected between replicates, whether in main effects or interactions, which could not be referred to error variation. The exception, line 7, gave a mean square of 0·9038 for differences between duplicates in all 7 components of the analysis taken together. When compared with the pooled error from the Or tests this is significantly high at the 1 per cent. level ; but since this was chosen as the most extreme mean square out of the five (from the 5 lines tested in replicate) it is doubtful whether its significance should be rated too highly. If pooled with the other four all significance is lost. The replicates therefore gave no reliable evidence of differing genetically. In what follows we shall therefore pool all replicates. Only in line 7 is there any possibility that this could lead to misjudgment.

In table 10 are set out the results of assay of all the lines tested including Or. The number of replicate assays is shown for each of them. The origins of some of these lines have not yet been described, but they will be included in the present consideration wherever it is necessary to complete the analysis. The analyses of variance show, as we should expect, that the lines are inhomogeneous in regard to the main effects of the 3 chromosomes. These results will be made the subject of more detailed examination below. The data on the second order interaction, X-II-III, give no indication that this is either significant when taken over all the lines or anything but the same in all of them. We can therefore neglect the possibility of any complex interaction involving all 3 chromosomes at once.

The three first order interactions are, however, more interesting. The lines taken together confirm the evidence from the Or analysis of a positive interaction between II and III. They also suggest that there is a negative interaction between X and II. Both these interactions seem to be homogeneous over all the lines when tested against the error variance from the Or assays, despite the failure to

TABLE 10

Results of chromosome assays on the twelve lines of the experiment

Line	Number of assays	Main effects			Interactions				
		X	II	III	X-II	X-III	II-III	X-II-III	
Or	11	0·79	0·54	−2·44	−0·15	−0·14	0·58	0·04	
Sk	4	−1·06	−1·30	−3·24	−0·67	−0·83	0·53	−0·00	
Line 1	1	0·25	−1·05	−3·42	0·54	0·60	0·42	−0·69	
Line 2	1	−2·30	−1·13	−3·25	0·10	0·21	0·34	−0·60	
Line 3	1	0·94	0·33	−2·20	−0·64	−0·29	0·27	−0·06	
Line 4	1	2·94	−0·03	0·77	−0·42	0·82	−0·20	−0·15	
Line 5	1	2·11	0·40	0·56	−0·06	0·67	−0·03	0·45	
Line 6	2	2·96	0·54	0·36	−0·26	0·95	−0·10	−0·15	
Line 7	2	2·75	3·01	0·66	−0·62	0·47	0·72	−0·42	
Line 8	1	2·62	3·23	1·72	−0·86	1·36	0·60	−0·58	
Line 9-1	2	1·94	3·18	1·18	−0·82	0·44	0·12	−0·02	
Line 9-2	2	2·96	2·76	5·65	−0·37	1·13	0·12	0·23	
Analyses of variance—Mean squares									N
Grand deviation		35·67*	15·86*	30·83*	3·32*	0·71	4·67*	0·02	1
Heterogeneity between lines		5·41*	5·57*	15·95*	0·26	0·99*	0·19	0·17	11

N = Degrees of freedom

* = Significant when compared with the error variance of 0·2869 from the Or analysis (table 9).

find that between X and II in the Or tests themselves. We may therefore describe the situation in another way. All the lines assayed show the same X-II and II-III interactions when compared with the tester. It would thus be more reasonable to say that in these lines X-II and II-III show no interaction but that in the tester they do so. In other words the interactions are the properties of the tester, possibly because of the marker genes the chromosomes carry there.

The interaction between X and III behaves differently. Unlike the other two there is little evidence to be obtained for this interaction from the grand deviation of all lines from the tester. But the lines themselves are not homogeneous in respect of it. Some of them must show it in one direction and some in the other. This interaction cannot therefore be referred wholly to the markers or indeed to any other genes of the tester.

Now the 11 lines can be divided into 5 groups according to the effects of selection. The first of these includes the parents, Or and Sk,

upon which selection has not been practised. The second includes line 1 and 2 which are both the outcome of low selection, and average about 34 chaetæ when both males and females are counted. The third contains only line 3, the original high mass line, which must be virtually at the Or level when allowance is made for the change in counting at S–13. The fourth group includes lines 4, 5 and 6, all of which come at the intermediate level of response to high reselection as described in the previous section, though line 6 approached the highest level of reselection in chaeta number by the time the assays were made. Finally lines 7, 8 and 9 (assayed at two different levels) were at the highest level of all, averaging for males and females between 52 and 56 chaetæ. If we consider the X-III interaction as it is distributed amongst these groups we find that the differences between the groups taken as wholes account for all but an insignificant residual of heterogeneity (table 11). The level of interaction is thus related to selection.

TABLE 11

Interaction of chromosomes X and III

Group	Average interaction (weighted)
Parents	−0·32
Lines 1 and 2	0·40
Line 3	−0·29
Lines 4, 5 and 6	0·85
Lines 7, 8 and 9	0·78

Analysis of variance	N	Mean squares
Heterogeneity between groups	4	2·2698 *
Heterogeneity within groups	7	0·2672

* Significant when tested against error variance from the Or analysis (table 9).

We can go even further. The parents have a negative interaction, and so does line 3 which has receded virtually to the parental level. The low lines have a high positive interaction and the two groups of high lines an even greater one. The negative interactions of Or and line 3 could be regarded as a positive interaction of the L as opposed to the T chromosomes for in these cases L departs from T in opposite directions in chromosomes X and III. The negative interaction of Sk cannot, however, be so interpreted as the departure from the tester is in the same direction in both X and III. It must thus be regarded as a genuine negative interaction ; and we may observe that it is higher than the more doubtful interactions of Or and line 3. There is thus some evidence that effective selection, whether for low or high chaeta number, appears to have produced a change towards positive interaction between X and III. That selection in either direction should do this is not surprising, for a positive interaction implies that the two sets of downward allelomorphs produce disproportionately low chaeta numbers when together as well as that the two sets of high allelomorphs jointly produce a disproportionately high chaeta number.

Nor is the lack of difference between these two high groups in the level of interaction surprising when we see that the difference in chaeta number between them depends largely on a change in chromosome II whose interaction is not in question. We are therefore led to conclude that selection may pick out sets of genes to some extent by their interactions as well as by their individual effects. The negative interactions of the parents, tending to reduce the sum of the individual effects of the genes on chaeta number, would thus be replaced by positive interactions tending to enhance the joint effects.

One last word about these interactions. If the scale upon which to represent the metric, here the chaeta number, is ill chosen interaction would appear for this reason alone (Mather, 1949*a*). Such interactions would, however, be uniform for all the pairs of chromosomes and would change uniformly as the metric changed in value. Neither of these criteria is satisfied in the present case: some pairs of chromosomes have interactions where others do not: and the X-III interaction is negative with the central values and positive with both extremes of the metric, low as well as high. Rescaling would not remove our interactions. It could only at most replace them by others requiring the same general interpretation. We have no reason to believe that the simple scale of chaeta number is not adequate to give a true picture of the genic interactions and their changes under selection, as well as of the main effects of the chromosomes, with which will of course be included the interactions between genes within the same chromosome.

So far we have barely touched upon what was the avowed purpose of the assays, to discover in which chromosomes the changes inferred from the responses to selection and reselection had occurred. The analyses of variance of table 10 show us that all three major chromosomes in the various lines differ in their main effects not only from the tester but also from one another. That they differ from one another is the important observation, for such differences in chaeta producing power cannot be ascribed to the major marker genes in which the tester differed from all the lines, but in which the lines, being wild-type, were all alike.

The effects of the major genes used as markers can be eliminated from the analysis by a further process of differencing. The Or X showed an excess of 0·79 in chaeta producing power over its counterpart from the tester, marked by *ClB*. The Sk X showed a corresponding deficiency of −1·06, the fact that it is a deficiency being indicated by the -ve sign. The Sk X can be compared directly with the Or and the effect of the common tester eliminated by subtraction. Thus if the chaeta producing power of the tester X is A, that of the Or O and that of the Sk S, we already know that

$$O-A = 0{\cdot}79 \quad \text{and} \quad S-A = -1{\cdot}06.$$

Then to express the power of the Sk X directly as an excess over

that of Or, so eliminating the effects of all genes peculiar to the tester, we need

$$S-O = (S-A)-(O-A)$$
$$= -1{\cdot}06-0{\cdot}79 = -1{\cdot}85.$$

The values so reached for all the chromosomes of all the lines are shown in table 12, which thus includes only comparisons between

TABLE 12

Effects of the individual chromosomes on chaeta number in females, expressed as excesses over those of the Oregon chromosomes

Line	Chaeta number of line females	Excess over Oregon (43·53)	Effects of			Total	Efficiency percentage
			X	II	III		
Sk	37·63	−5·90	−1·85	−1·83	−0·80	−4·48	76
1	36·00	−7·53	−0·54	−1·59	−0·98	−3·11	41
2	37·04	−6·49	−3·09	−1·67	−0·81	−5·57	86
3	44·35	0·82	0·15	−0·21	0·23	0·17	21
4	50·65	7·12	2·15	−0·57	3·21	4·79	67
5	52·26	8·73	1·33	−0·14	3·01	4·20	48
6	52·60	9·07	2·17	0·01	2·80	4·98	55
7	59·53	16·00	1·96	2·47	3·10	7·53	47
8	63·35	19·82	1·83	2·69	4·16	8·68	44
9-1	59·19	15·66	1·15	2·65	3·62	7·42	47
9-2	70·25	26·72	2·17	2·22	8·09	12·48	47
	Sums neglecting sign	123·86				63·41	51

wild-type chromosomes, taking the Or chromosomes as the standard of reference in all cases. The changes in chaeta producing power under selection are thus not to be referred to any known major genes. They depend on alterations in a polygenic system which involves at least all three large chromosomes.

Table 12 tells us a number of things. The Sk parent has a lower chaeta number than the Or because of the action of genes in all chromosomes. The result from the low mass line (line 1) whose origin was described in section 3 (*a*) is a little disappointing since its chromosome appears to have much the same effects as those of the Sk parent, except in the X where the effect seems to be to produce more chaetæ in line 1 than in Sk. Caution is, however, engendered when we look at the total effect accounted for by all the three chromosomes together. In Sk this is −4·48 or 76 per cent. of the observable difference in mean chaeta number between Sk and Or females (table 12); but in line 1 it is only 41 per cent. For some reason the efficiency of the assay of line 1 is only half that of Sk, so that comparisons of the effects directly traceable to the chromosomes by the assay must be somewhat unreliable. This question of efficiency will be discussed further below.

Most of the assays show a reasonably uniform efficiency. The assay of line 2, whose origin is yet to be described, is of remarkably high efficiency with 86 per cent., but we are not specifically concerned with this line at present. We need only note that at least its X has a lower chaeta producing power than those of the two parents. Line 3, the first high mass line of the previous section, has a low assay efficiency of only 21 per cent.; but this is doubtless merely a reflection of the fact that both its overall chaeta number and the summed effects of its 3 chromosomes approximate closely to those of Or. With such small differences sampling variation will sway the percentage to a very marked degree. The chief point to notice about this line is that so far as the assays go they agree well with the inference, made in the last section, that its chromosomes must closely resemble those of Or in effect on chaeta production. That they are not, however, genetically identical will become clear in section 4 (*a*).

The rest of the assays with which we are concerned at present, those of lines 4, 6, 7 and 8, have reasonably similar efficiencies and comparisons can therefore be made with some confidence. Lines 8 and 7, the first high reselection and the mass line from it, exceed even the higher of the two parents, Or, in all 3 chromosomes. Furthermore they are much like one another, line 8 only exceeding line 7 by a little in chromosome III. This excess may be due to a delayed response which had occurred in line 8 prior to the assays, and by which it differed a little from line 7 after S–84. Lines 4 and 6, the seventh reselection which went up only half way and the mass line from it, closely resemble line 7 in chromosome X and III, but in chromosome II they are like line 3 and the Or parent. Thus the change in the high mass line between S–27 and S–30 was in chromosome II. The high chaeta combinations of this chromosome had vanished so that reselection could only pick out those in X and III from S–30 onwards. The advance under reselection must thus be smaller than the advances obtained earlier, though the reduction in the advance obtained was perhaps a little greater than would be expected from consideration of the assays alone. We do not, however, know the efficiency of the assays for the individual chromosomes, and it may be that it is lower for II than for X and III, so that the difference seen in the assay for this chromosome would represent a smaller fraction of that which was actually being produced between the lines themselves. However, this may be, the assay results bear out the conclusions reached from the consideration of the results of selection and reselection as well as could be expected.

One last point about the assays requires comment. We have already seen that their efficiencies will depend on the dominance relations of the genes involved relative to the tester, and on the amount of residual recombination with the tester. The effect of the latter factor has been estimated as an average loss of 10 per cent. in efficiency. The remaining loss must be due to dominance, either by it being

incomplete in the individual tester genes as compared with their allelomorphs from the lines, or by it being balanced in that it goes one way in some of the tester's genes and the other way in the rest. Low efficiency would mean low dominance of the tester's genes. With absence of dominance, or with fully balanced dominance, the loss from this cause would be 50 per cent., which taken with the recombinational loss would give 45 per cent. efficiency in the final results.

It is impossible to test the efficiency of the assays by direct comparison with the tester as we cannot obtain flies homozygous for the actual tester chromosomes used. There is thus no means of measuring the overall difference in chaeta number between tester and line flies. The indirect comparisons of the various lines with Or suggests, however, that in the assays of both Or and the majority of the lines efficiency must have been near to this 45 per cent. A few lines, notably Sk and line 2, depart somewhat widely from this expectation and there is consequently some ground for thinking that the dominance relations of their genes relative to the tester's were different from those of the other lines. On this view the Sk and line 2 genes must have been recessive to their allelomorphs from the tester more often than not.

(d) Crosses between the levels

The high mass line (line 3), and the first (lines 7 and 8) and seventh (lines 4 and 6) reselections from it, establish three levels of response to selection for high chaeta number, at which the lines can remain stable or virtually so in respect of chaeta number even under the conditions of mass culture. These levels, which can be seen clearly in figs. 1 and 2, will be designated as the upper (U), middle (M) and lower (L). The chromosome assays have shown us that U differs from M in respect of the polygenic combinations carried in chromosome II, and from L in respect of the combinations of all three large chromosomes. M differs from L in the combinations of chromosomes X and III. The properties of these combinations in potence, *i.e.* the integrated dominance of all the genes in the combination (Wigan, 1944), and segregation were further investigated in crosses between the levels.

The first of these crosses, between U (line 7) and M (line 6), was made with flies taken from S–58. The flies used as parents for this cross, and indeed for all the crosses between levels, were taken as having chaeta numbers closely approximating to the average for the sex in question in the particular culture. The cross was made reciprocally, two matings being made each way. The average number of chaetæ for the two sexes taken over all four F_1 cultures, was 50 which is almost exactly midway between the parent values (fig. 4), and the average of the sexes taken over both F_2's, from the

reciprocal F_1's, was 51½. It will be seen from fig. 4, however, that the chaeta numbers of both the parental lines were higher in S–60,

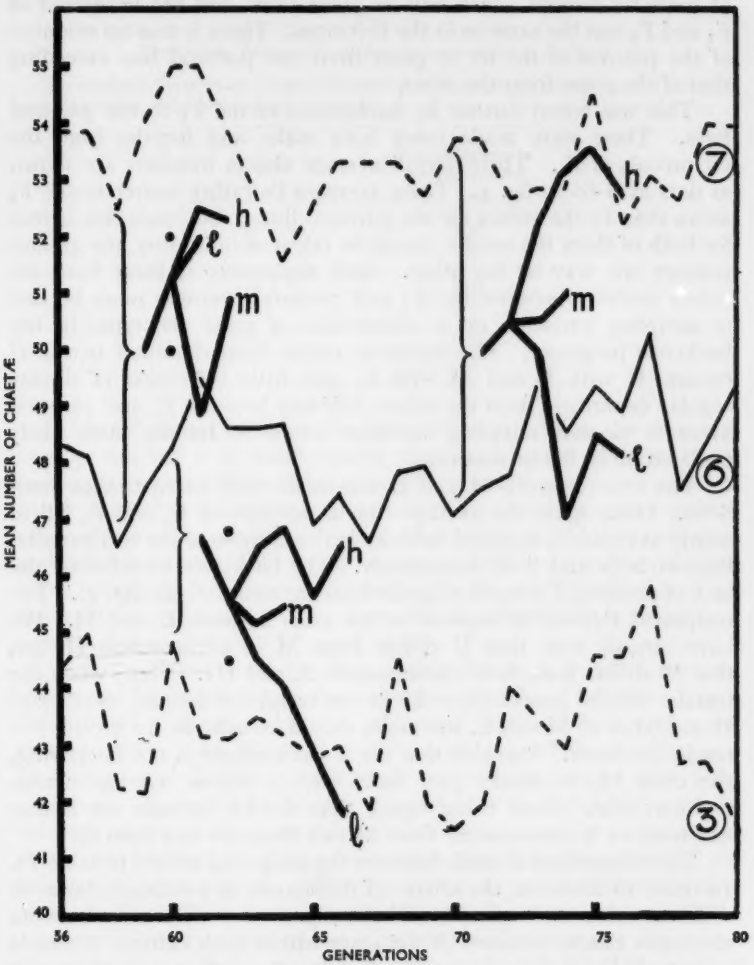

FIG. 4.—The level crosses between lines 6 and 7, lines 3 and 6, and their behaviour under selection. The brackets join the lines at the generations from which the parents are taken. The F_1 means are at the beginnings of the heavy lines near the points of the brackets. The heavy lines proceed to the F_2 means, after which they diverge into the three selected lines, for high (*h*), mid (*m*) and low (*l*) cheata numbers. These lines were all terminated deliberately. The large dots indicate the means of the backcrosses. The parental lines are shown faintly.

when the F_2 was raised, than in S–59, due doubtless to non-heritable changes. Thus the F_2 mean lies midway between those of the parent

lines, so that it resembles the F_1 in mean chaeta number as closely as would be expected. The cross between U and M was repeated at S–70 for reasons which will be given later, and the behaviour of F_1 and F_2 was the same as in the first cross. There is thus no evidence of the potence of the set of genes from one parental line exceeding that of the genes from the other.

This was tested further by backcrosses of the F_1 to the parental lines. These were made using both males and females from the reciprocals of F_1. Their overall average chaeta numbers are shown as dots at S–60 in fig. 4. These averages lie rather nearer to the F_2 mean than to the values for the parental lines ; but since this is true for both of them the results cannot be taken as indicating any greater potence one way or the other. Such departures as these from the points midway between the F_2 and parental averages must be due to sampling variation or to elimination of some genotypes in the backcross progenies. The backcross results from the other two level crosses, U with L and M with L, give little indication of similar regular departures from the values half-way between F_2 and parental averages so that sampling variation seems to be the most likely explanation in the present case.

The cross between M and L was made with parents taken from S–60. Once again the average chaeta numbers of F_1 and F_2 fell as nearly as could be expected midway between those of the two parental lines in S–61 and S–62 respectively. The backcrosses confirmed the lack of potence of the sets of genes from the two parents (fig. 4). The reciprocal F_1's are of interest in this cross between L and M. We have already seen that U differs from M in chromosome II, but that M differs from L in chromosomes X and III. Thus, while the females will be genetically alike in the reciprocal crosses, whether of U and M or of M and L, the males should be alike in the former but not in the latter. Provided that the Y chromosome is not interfering, the cross M×L should give males with a higher average chaeta number, other things being equal, than L×M, because the former will have an X chromosome from M and the latter one from L.

The comparison is made between the reciprocal crosses in table 13. In order to minimise the effects of differences in conditions between different cultures, the data have been expressed as differences between the mean chaeta numbers of the sexes within each culture, the male average being deducted from the female. Any effect common to the flies of one culture will thus be eliminated. Since several cultures were raised of each reciprocal of each cross, the excesses of females over males were found for each culture and then averaged over all like cultures. The figures so obtained are given in the table. Where the X chromosomes of the parents differed in chaeta producing power the excess of the female chaeta number over the male should be lower where the female parent had the higher chaeta number. Thus the value in table 13 for M×L should be lower than that for L×M by

an amount reflecting the difference in effect on chaeta number of the X chromosomes of M and L.

TABLE 13

Comparisons between the reciprocal crosses between the levels

The entries in the table are the sex differences in chaeta number (female—male) averaged over all cultures of the given types.

Cross	Sex difference in chaeta number
U × M	8·16
M × U	8·31
M × L	5·92
L × M	6·21
U × L	6·00
L × U	7·55

The difference between the two reciprocals is in the direction anticipated, but it is much smaller than might have been expected. In the assays the difference between lines 3 and 6 was about 2 chaetæ in the X, and as we saw the assays will not generally measure the full effect of the chromosome. Instead of a value of this order we find a difference of only $6{\cdot}21 - 5{\cdot}92 = 0{\cdot}29$. True it is larger than the value obtained from the reciprocals U×M and M×U, where no difference is expected to arise from the X chromosomes; but it is nevertheless too small.

A further estimate of the effect of this difference in the X chromosomes can be obtained from the cross between U and L levels. These differ in all 3 chromosomes, the difference in the X being the same as that between the X's of M and L (table 11). This cross between U (line 7) and L (line 3) was made twice, with parents from S–64 and S–74. Again the mean chaeta numbers of F_1 and F_2 fall midway between those of the parental lines on both occasions; and back-crosses made from the first cross confirm the lack of potence of the two sets of genes (fig. 5). We may note that, just as with the crosses between U and M and between M and L, these results will be unaffected by any effects of the X in reciprocal crosses, for the values shown in fig. 5 are the averages of cultures among which the various reciprocals were equally represented.

The effect of the difference between the X's on the reciprocal F_1's from the cross U and L levels is shown in table 13. It is 1·55, a value much larger than the estimate obtained from the crosses between L and M, and one more in keeping with the difference of two found in the assays. It would thus appear that the low difference found from L and M was in part due to the vagaries of sampling variation. The sex difference fluctuates sufficiently between cultures

of comparable crosses easily to make possible a difference as great as that between these two estimates of the effect of the X. Nevertheless even the higher estimate is somewhat below that obtained from the assays, which were themselves very unlikely to have revealed the whole of the difference.

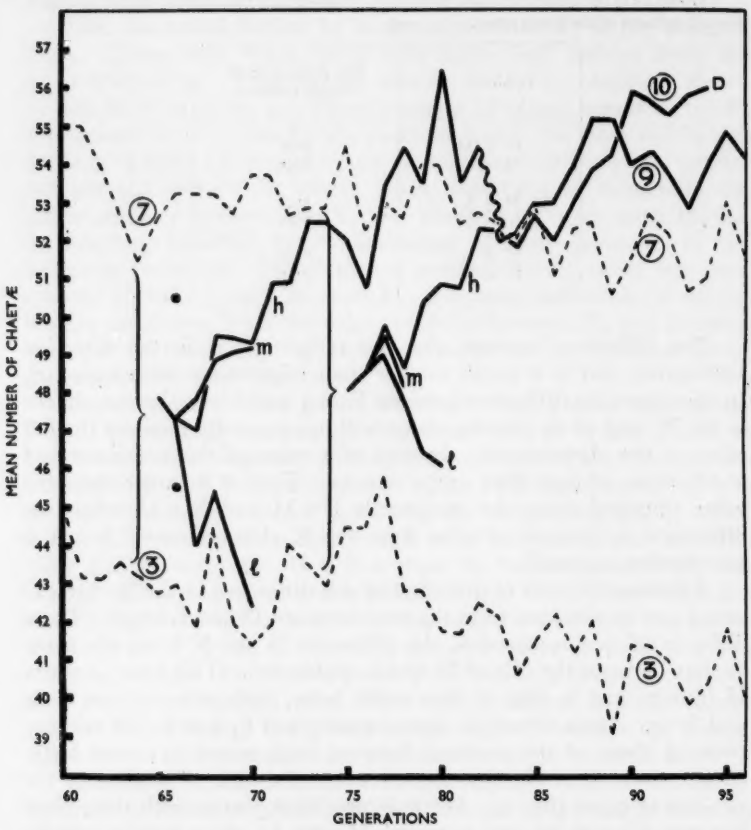

FIG. 5.—The level crosses between lines 3 and 7 (shown faintly). The symbols are as in fig. 4. Line 10 died out through sterility. The *l* and *m* lines were terminated deliberately.

Thus the comparison of reciprocal crosses shows differences in the places where they would be expected, but of a size less than would be expected. They suggest that the Y chromosome may have been acting to obscure part of the effect of the X. If line 3 had a Y chromosome as well as an X whose effect was to determine a lower chaeta number than did the corresponding chromosome of line 6, or of course of line 7, the difference between the reciprocals would

represent not the full effect of the difference between the X's but only the excess in effect of the difference in the X's over that of the Y's. That the Y can affect the manifestation of chaeta characters is known from earlier work (Mather, 1944), and it also seems likely that its differences will generally be smaller than those of the X (Wigan, 1949), in the way that the present data would require.

The data from the F_2 and backcrosses of these crosses between the three levels can be put to use in another way. They can be made to yield estimates of the number, not of genes but of effective factors, *i.e.* groups of genes effectively behaving as units in transmission (Mather, 1949*a* and *b*), in which the lines at the various levels differed. Four assumptions must be made in order to obtain the estimates (Wright, 1934). The factors must be assumed: (i) to be isodirectionally distributed between the parents of the cross, *i.e.* for the + or increasing allelomorphs of all of them to be in one parent and the − or decreasing allelomorphs in the other, (ii) to be of equal action, (iii) to show no dominance, and (iv) to be unlinked. Failure in any of these respects must lead to an underestimate.

Using the notation of Mather (1949*a*), let the difference in manifestation of the character, *i.e.* in chaeta number, between individuals of the two genotypes which differ only in being homozygous for the allelomorphs of factor $A-a$ be $2d_a$ and let the chaeta number of the corresponding heterozygote, *Aa*, depart from the average of the values of the two homozygotes by h_a, which can take a + or a − sign according to the direction of departure. Then this gene contributes $\frac{1}{2}d_a^2+\frac{1}{4}h_a^2$ to the variance of F_2. In the absence of linkage we can put $D = S(d^2)$ and $H = S(h^2)$, summation proceeding over all the factors which contribute to the difference between the parents, and the variance of F_2 becomes

$$V_{F_2} = \tfrac{1}{2}D+\tfrac{1}{4}H+E$$

where E is the non-heritable component.

Now if $d_a = d_b \ldots = d_k = d$ for all the k factors concerned in the segregation, the parental difference in chaeta number will be

$$\bar{P}_1-\bar{P}_2 = 2S(d) = 2kd$$

where the factors are distributed isodirectionally. At the same time

$$D = S(d^2) = kd^2$$

whether distribution is isodirectional or not. Then with isodirectional distribution

$$\frac{\frac{1}{2}(\bar{P}_1-\bar{P}_2)^2}{D} = \frac{(kd)^2}{kd^2} = k$$

But when the distribution is not isodirectional

$$\bar{P}_1-\bar{P}_2 < 2S(d)$$

and k must be underestimated.

When the various d increments are not equal and we put $kd = S(d)$ so that

$$d_a = d(1+a_a),\ d_b = d(1+a_b),\ \text{etc.},$$

we find

$$\frac{\frac{1}{2}(\bar{P}_1-\bar{P}_2)^2}{D} = K = \frac{k}{1+V_\alpha}$$

where V_α is the variance of a and K must thus be less than k.

The calculation as shown requires the separation of the D and H components of V_{F_2}; but this cannot be undertaken from F_2 data alone. If we estimate E directly from the parents and F_1, and deduct it from V_{F_2} we are left with $\frac{1}{2}D+\frac{1}{4}H$. This will afford a consistent estimate of D only where H is 0, *i.e.* in the absence of dominance. Otherwise $\frac{1}{2}D+\frac{1}{4}H > \frac{1}{2}D$, and the use of twice the heritable variance of F_2 as an estimate of D must lead to underestimation of k by an amount which will reflect the dominance relations of the factors as these are expressed in H.

With isodirectional distribution of the factors between the parents any linkage must be in coupling. In that case $D > S(d^2)$ by an amount depending on the recombination values and relative magnitudes of the gene effects (Mather, 1949*a*). This must again lead to an underestimate of k.

A test of dominance is possible where, as in the present case, backcrosses are available as well as F_2's. The summed variances of the two backcrosses, one to each parent, are (Mather, 1949*a*)

$$V_{B_1}+V_{B_2} = \tfrac{1}{2}D+\tfrac{1}{4}H+2E$$

Then after deducting the non-heritable components as estimated directly from the variances of the parents and F_1, the heritable portion of

$$(V_{B_1}+V_{B_2})-V_{F_1} = (\tfrac{1}{2}D+\tfrac{1}{4}H)-(\tfrac{1}{2}D+\tfrac{1}{4}H) = \tfrac{1}{4}H.$$

In the present case taking all three level crosses into account, the sum of the summed backcross heritable variances is actually less than the sum of the heritable variances of the three F_2's, *i.e.* our estimate of the summed values of the three H's is negative. Now H, being the sum of quadratic quantities, cannot be negative, so that we must take 0 as the best estimate of the summed H values. Or to put it another way, we can safely assume that dominance is absent, or at least on the average so small as not to be a disturbing factor in the calculations. The lack of potence, which is dominance integrated over all the factors in question, as already observed agrees with this conclusion.

Now if we take $H = 0$, then

$$V_{B_1}+V_{B_2}+V_{F_1} = \tfrac{1}{2}D+2E+\tfrac{1}{2}D+E = D+3E.$$

Deduction of three times the average variance of parents and F_1,

which affords the best available estimate of E, will give an estimate of D. This is done for all three level crosses in table 14.

TABLE 14

Estimation of the numbers of effective factors in the level crosses

Cross	Average variance of parents and F_1 (= E)	Sum of variances of two backcrosses and F_2 (= D+3E)	D	$\frac{1}{4}(\bar{P}_1-\bar{P}_2)$	K
U × M	9·82	33·11	3·65	2·5	1·71
M × L	7·93	27·07	3·28	2·5	1·91
U × L	8·00	34·28	10·28	5·0	2·43

The estimates of E were found as the average variances of the chaeta number in the two parental lines, in the generations from which the parents of the F_1's and of the backcrosses were taken, and in the F_1. In so far as the parental lines were not fully homogenic, this estimate will include a small heritable component; but one which, we may observe, has nothing to do with the differences between the levels, and which may therefore be fairly eliminated with the non-heritable component of variation. In the case of the crosses of U and M and of U and L, which were made twice, the average was taken over the various families of both crosses. These estimates of E appear in the second column of table 14. The third column of the table gives the sums of the variances of the two backcrosses and F_2 in the various level crosses. In all cases the variances of each backcross, and of F_2, were averaged over all families of the particular type, so removing any effect of differences between reciprocal crosses. It should also be noted that in all cases the variances were found for males and females separately within each culture, the two then being averaged so as both to eliminate the inflationary effect of the difference in sex means and to make equal adjustment in all types of family for any small differences there might be between the sexes in variance. D was then found by subtracting three times the entry in column 2 from that in column 3 of the table. Thus for U × M,

$$D = 33{\cdot}11 - 3 \times 9{\cdot}82 = 3{\cdot}65$$

and so on.

The differences in mean chaeta number between the parental lines were not found using the mean chaeta numbers of these lines in the generations from which the actual parents were taken, because presumably under the action of the environment these means fluctuate from generation to generation sufficiently to introduce error into the calculation. Instead the average chaeta numbers of the three parental lines were estimated over the relevant ranges of S–56 to S–80 by eye from fig. 4, as 53·25, 48·25 and 43·25. Then half the parental differences are as shown in column 5 of table 14. Each of these

values squared and divided by the corresponding value of D gives K, the estimate of the number of effective factors k, as shown in the final column of the table.

What do these values of K tell us? We have already discovered from the assays that U differs from M in chromosome II and from L in all three chromosomes, while M differs from L in chromosomes X and III. If these chromosomes or the parts of them carrying the differences were themselves behaving as effective factors we should expect $K = 1$ for $U \times M$, $K = 2$ for $M \times L$, and $K = 3$ for $U \times L$. The values obtained are in fact of this order of size and they fall in the same sequence. That of 1·91 for $M \times L$ is a very close approximation to the expected 2, but at 1·71 that for $U \times M$ is somewhat high, and at 2·43 that for $U \times L$ is somewhat low. When, however, we recall that even in the cross of U and L the heritable portion of $V_{B_1} + V_{B_2} + V_{F_2}$ is less than half the non-heritable, so making the error variance of D and hence of K disproportionately high, and that error variance is made all the larger by the experiment not being designed and conducted in the way best suited to yield data for the calculation of K, these differences between the values observed and those expected are set in their proper perspective. The overall agreement in magnitude and sequence is very good, and the departures in detail are reasonably ascribable to error variation. It would thus appear that just as, once built up, the linked polygenic combinations within the chromosomes behave as effective units in selection and in loss within the lines, so they also behave as effective units in the segregation which follows crossing between lines. It would further appear that the combinations cannot cover a great genetic length within the chromosomes, for if they did it would be difficult to see how they could behave as units, at least in transmission through females.

On this view, that the differences within each chromosome are behaving as one unit in segregation, all the assumptions made in regarding K as a consistent estimate of k will be satisfied. Distribution must be isodirectional—selection has seen to that. The factors must also, of course, be unlinked, for they are in different chromosomes. And the effects of the different chromosomes, as measured by the assays, are approximately equal. Table 12 shows us that the difference in the X is about 2, that in II about 2·5, and that in III about 3. Then in the cross of U and L the average of d is 2·5, and $a_X = -0{\cdot}2$, $a_{II} = 0$ and $a_{III} = 0{\cdot}2$. Thus $V_a = \frac{1}{2}(0{\cdot}08) = 0{\cdot}04$, which will cause little disturbance in the estimation of the number of effective factors. With the effects of dominance we have already dealt. Everything points to the operative parts of the chromosomes having behaved as effective factors in the segregation following the level crosses.

If the chromosomes behave as effective units in segregation, they should, in the absence of disturbing agencies, also behave as units in selection from the F_2's. Such selection tests were in fact made

(figs. 4 and 5). Three lines were taken off each F_2, one (h) in which the parents were selected in each generation for high chaeta number, one (l) in which they were selected for low chaeta number, and a third (m) in which they were selected as having chaeta numbers near the mean. The first such test was made from the F_2 of the cross between U and M at S–60. In two generations the h line had gone far towards recovering the chaeta number of the U parent. The other two lines, however, behaved strangely in the first generation of selection, l staying in the middle, as m was expected to do, and m falling as l was expected to do. Continuing the putative m line, by breeding from flies with central chaeta numbers, resulted in its average number rising almost back to the F_2 mean in the next generation (fig. 4). The suspicion that l and m had been accidentally interchanged in the first generation of selection thus became too strong for any reliance to be placed in the results, and the test was abandoned in favour of starting again with a fresh cross of U and M.

This was made at S–70 and the three selection lines were once more taken off the new F_2. The mean chaeta number of the m line so obviously stayed near the F_2 mean that this line was terminated after two generations. Meanwhile both h and l had regained, in fact in the latter case transgressed, the upper and lower parental levels respectively. After the first two generations they each maintained a constant relation with the corresponding parental line, following the vagaries of these lines in a most striking fashion. Clearly, as would be expected with only one effective unit of segregation, selection had quickly restored the parental genotypes. That l fell below the lower parent is in no way surprising because, as we shall see in the next section, line 6 was not wholly homogenic, so that its chaeta number represents the mean action of the combinations it carries. Any particular sample of these such as would be recovered in a selection from an outcross might well show a small constant departure from the average of the full group.

Selection from the cross between M and L gave basically similar results (fig. 4). Again the m line stayed near the F_2 in mean chaeta number, while h and l quickly reached mean chaeta numbers approximating to those of the M and L parents, after which they maintained a constant relation with those lines. As in the cross of U and M, the means of the h and l lines departed a little from those of the parental lines in the way we might expect. One point is, however, worthy of note. In selecting from the cross of U and M, h and l nearly recovered the parental levels after one generation's selection, and passed them slightly after two. Following the cross of M and L, although h achieved its full advance in two generations, the l line took three to reach the lower parental level. Now the total response was much the same after both crosses, but in that of M and L it had to be achieved by the joint selection for two effective factors (one sex-linked) as opposed to one. Instead of $\frac{1}{4}$ at most $\frac{1}{8}$ of

the flies would be of the correct genotype. Thus with 10 per cent. of the flies selected as parents and the expressions of the genotypes obscured by a high measure of non-heritable variation, progress under selection is likely to be slower from the cross between M and L than from that between U and M.

This statement is, however, one of probability only. If the extreme genotype should be picked up in selection in spite of its relative rarity, then progress must be rapid. This seems in fact to have happened in the first cross between U and L (fig. 5). Despite the fact that three effective factors were segregating, so that at most $\frac{1}{35}$ of the flies would have the extreme low genotype in F_2, most of the progress in the l line was already accomplished after one generation's selection, and it was complete after two. The h line, on the other hand, progressed very slowly ; so slowly in fact that it took 7 generations to approach the upper parental level at all closely, and 10 generations to pass it. Such progress is much slower than would be expected even where three effective factors have to be sorted out. Two steps were therefore taken to see further into the matter. The h line was itself kept going as line 9, and the whole cross was repeated.

In the repetition of the cross the new l line displayed the same slow response that the h line did in the first. Evidently the fortunate chance of early selection of the extreme low genotype, responsible for the rapid advance in the first l line, had not repeated itself in the second. This second l line was, however, discontinued once its general behaviour had become clear. The h line (maintained as line 10) from the second cross also showed a slow advance, crossing the parental level after 8 generations' selection—a result sufficiently well in keeping with the behaviour of line 9.

The subsequent behaviour of lines 9 and 10 is of great importance for our interpretation of their early progress. Neither of them stabilised near the parental level in the way that the selection lines showing rapid early advance had done. Line 9 hovered between 1 and 2 chaeta above its parent line 7 for 5 generations under selection but then owing to sterility causing a complete failure of the cultures in one generation it had to be recovered without selection from the old culture at S–83. This resulted in a decline of the chaeta number to the parental level, after which, however, it rose again under selection till it exceeded the parental line by about 2 chaetæ. Here it remained constant for some 25 generations in spite of a somewhat low fertility. Line 10 progresses fairly steadily, apart from 4 generations near stability at about the parental level, until it eventually came to exceed the parent line by 4 chaetæ. At this point its fertility became so poor that it died out in S–94.

Thus not only did line 9 and 10 progress more slowly than expected under selection ; they progressed well beyond the parental level, and they developed a sterility which was foreign to the parent. They appear in fact not to represent mere recoveries of the parental genotype,

but to owe their high chaeta numbers to the building up of new combinations by crossing-over. These would take time to develop, so that progress under selection would be slow; they would not exactly match the old ones in effect on chaeta number, so that the parental level could be exceeded; and they would carry with them the customary correlated response of lowered fertility. If this were all the evidence, it might still be considered that the data were not conclusive. Fortunately a second correlated response to selection for chaeta number was discovered in the form of changes in the number of spermathecæ of the females; and, as we shall see in section 4 (*a*), lines 9 and 10 both showed spermathecal types not found in their parent lines. There can thus be no doubt that they carried new combinations, and that their tardy progress under selection was due to the slow building up of these by recombinations within the chromosome, just as had occurred in the original high selection line before S–20.

Line 10 died out by reason of its sterility before its chromosomes could be assayed; but assay results are available from line 9. Those given under the heading of line 9-1 are the more relevant set, line 9-2 covering assays made after a delayed rise which occurred approximately at S–112 (fig. 3). So far as can be judged from the assays, chromosomes X and II had much the same chaeta producing power in line 9 as in lines 4-8. Line 9-1 is rather low in the X but the results from 9-2 suggest that this may have been due, in part at least, to error variation (table 12). In chromosome III however 9-1 exceeds all the earlier lines except 8, which had itself shown, before it was assayed, a delayed rise above the level which it had earlier shared with line 7. It would appear therefore that line 9 mainly owed its difference in chaeta number from its parent line 7 to chromosome III, there being also a possible smaller difference, in the other direction, in the X. It is thus of interest that, as we shall see, chromosome III is most likely responsible for the altered effect on the spermathecæ. Evidently new combinations have been built up by selection at least in chromosome III.

As to why high selection from the crosses between U and L should have resulted in the slow building up of new combinations rather than the speedy recovery of the high parental genotype we can only conjecture. Possibly individuals having this extreme genotype, though viable in isolation, were at so marked a disadvantage in competition with those having the less extreme and more heterozygous genotypes in the F_2 that the extreme type persistently escaped selection. Progress could then be made only as new combinations were built up from the heterozygotes. Against this view is the fact that the ultimate outcome of the selection was less viable or less fertile than the individuals of the parental type, though again this might not be so serious a difficulty for the argument if the decline in fertility could have been both gradual and matched by an equally gradual replacement of the more vigorous types. However this may be, the results

are clear in the main respect. Selection can at times fail to seize on a favourable type which segregation must have been capable of producing, and which is known to be sufficiently viable and fertile to persist in the absence of competition. Progress must then depend on building up new combinations once again, with all the attendant complications and disturbances from correlated responses.

(e) *The relation between chaeta number and fertility*

A number of facts about the relation between chaeta number and fertility have emerged from the account already presented. Fertility fell in both the high and low selection lines taken off the original F_2. The fall was reasonably steady in the high line, just as was the rise in chaeta number. In the low line the fall in chaeta number was more erratic, there being something of a pause in the response before S–20, after which the fall was resumed and accelerated. The corresponding behaviour of fertility can be seen in fig. 7. Here too there was a pause, even a recovery of fertility, before S–21, at which generation the downward trend was resumed and became very rapid until the line died out at S–35.

In the high selection line the relaxation of selection resulted in fertility taking charge as the capital character, so that the chaeta number of the mass line fell back to an average of little more than 40, at which it became more or less stable. In all seven of the reselections from this mass (line 3) the fertility was lower after three generations of selection than it was in the first generation. It would thus appear that the first effect of reselection was to cause something of a fall in fertility within the line, though the 7 lines differed widely in fertility among themselves in no apparent order.

Only two of the reselections were continued over a sufficiently long period for their later behaviour to appear, *viz.* the first one which became line 8 at the upper level, and the seventh which became line 6 at the middle level. In line 8, which recovered the high chaeta number attained in the first high selection only at the expense of almost complete sterility in S–20, fertility was, apart from a peak at S–28, poor right up to S–31, dangerously poor in fact on several occasions (fig. 7). After S–31 however a rise set in, unmatched by any corresponding change in chaeta number, until S–41. After this there was again a decline, followed in its turn by a fluctuating behaviour which lasted more or less throughout the experiment. A small general decline at about S–83 probably has little genetical significance as it seems to have been shown by all lines. The important feature about line 8 is that its fertility did recover sufficiently for the line to be maintained at a time when chaeta number was itself stable. The fertility from S–43 onwards was in fact on the average at least $\frac{3}{4}$ that of the original high selection in its early generations, and was above that of generations S–15 to S–20.

Fertility in line 6 fluctuated somewhat, but it was never dangerously low. Up to S–70 its average was higher than that even of the early generations of the initial high selection, when chaeta number was well below the middle level upon which line 6 fell.

As already recorded, mass lines were made up from these two reselection lines. Mass line 7 was taken off selection line 8 at the upper level, and mass line 4 from selection line 6 at the middle level. These two mass lines were stable from the outset in their average chaeta numbers, hardly changing in a hundred or more generations. They did not repeat the early behaviour of line 3, only copying its later stability. Such immediate stability could come about in two ways. It might be because the selection line, from which the mass was begun, was itself homogenic, so that no matter what system of breeding was adopted change could come about only as mutation gave rise to new variability. Or it might be because, though heterogenic, the combinations giving the chaeta number in question had a selective advantage in mass culture over any which would bring about a change in chaeta number. Such an advantage must lie in a higher fertility, in the broad sense in which we are using this term.

A decision has been reached between these two alternatives by back-selection tests. Selection lines for lowered chaeta numbers were taken off lines 3 and 6 at S–47 and S–48 respectively (fig. 1). In both cases a response was obtained to the selection, that of the back-selection from line 6 being the greater. These lines were terminated at S–59. Thus the mass line 3 had within it genes capable when recombined of giving combinations reducing chaeta number. Mass line 4 was not itself tested, but since line 6, from which it was taken, also carried genes capable of building up low chaeta combinations, there is little reason to doubt that line 4 too would carry the same heterogenicity.

The test of mass line 7 did not admit even of this slight doubt, for the back-selection (line 2) was taken from the mass itself at S–56, 7 generations after it had been made up from the selection line 8. As fig. 6 shows, the response to low selection was immediate and steady up to S–67, by which time the average chaeta number had fallen to the middle level as a result of change in chromosome II (table 12). There it stayed until S–83 when another and longer fall began so that by S–90 line 2 was nearly down to the level of the low mass line 1, from which it finally became indistinguishable in chaeta number at S–107. All 3 large chromosomes had changed in their chaeta producing power by this time. A high selection (line 5), taken from line 2 at S–81, failed to advance, maintaining the middle level until its termination at S–110. A further low selection, taken from line 5 at S–100, showed no change for 6 generations, after which it was terminated.

The mass line 7 was thus stable in chaeta number in spite of its containing all the genes necessary to reduce the chaeta number below

the original parental level, when they were brought together by recombination under selection. Combinations tending to reduce the number must continually have been occurring in the mass, yet they never obtained a hold in it. This failure cannot have been due to the method of maintaining the mass line. In both mass and selection line 20 flies of each sex were taken for counting. In the first selection 4 of each sex, and in later selection generations 2 of each sex from each of 2 cultures, were chosen to be the parents of the next generation. For the mass, 10 of the 20 were taken without selection from each sex for use as parents. Thus any fly which could be chosen in starting

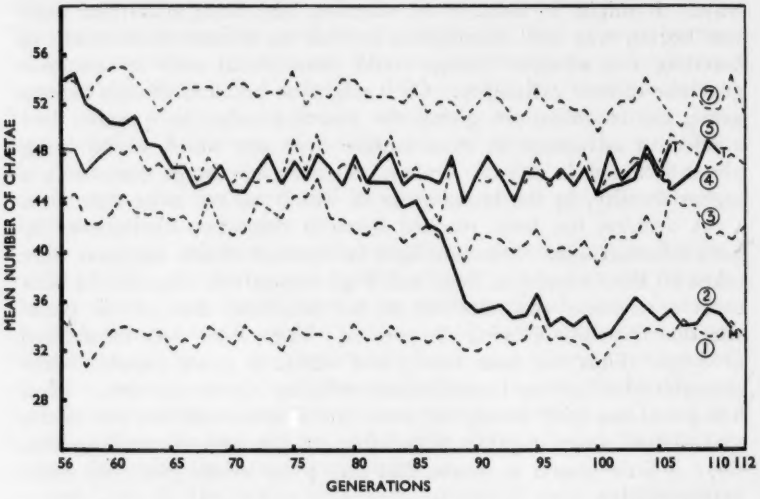

FIG. 6.—The origin of line 2 by low selection from line 7, and its subsequent behaviour. Line 5 is a high selection from line 2. Line 5 was separated into high and low selection lines at S-100 these being terminated deliberately at S-110 and S-106 respectively. The letter M indicates that at S-111 line 2 was mass cultured and continued as such in later generations (see fig. 3).

the selection line would have a half chance of being used in the mass line. Since flies with genotypes capable of giving lowered chaeta numbers obviously occurred in the mass line, for selection would have been ineffective in their absence, they could hardly have been missed as parents generation after generation in the mass itself. It can only be supposed therefore that in line 7 the low chaeta combinations were at a selective disadvantage under the conditions of mass culture as compared with those giving what may be termed the standard range of chaeta numbers.

Such a disadvantage of the low chaeta combinations need of course be neither unconditional nor final. It only need be sufficiently enduring to prevent the immediate combinations from obtaining a foothold.

It is not necessary to suppose that combinations built up by recombination from these, and giving still lower chaeta numbers (and the slow rate of fall as well as the chromosome assays show that change did depend on recombination), had an equal incubus of poor fertility ; for by virtue of the selective elimination of their progenitors these

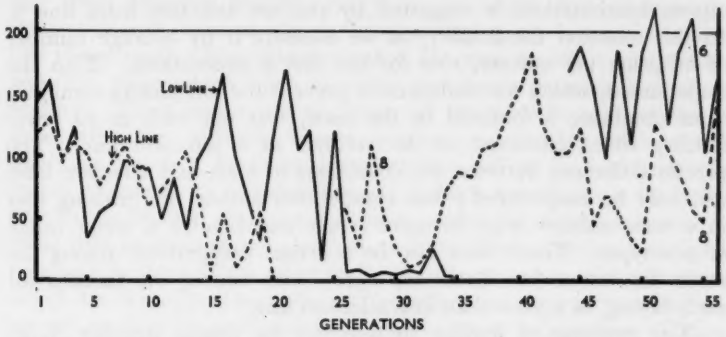

FIG. 7.—Fertility as measured by mean output of flies per culture in the original high and low selection lines and in lines 6 and 8 up to S-56. Note that all these are selection lines : the broken lines do not indicate mass mating as in earlier figs. Ordinate is mean number of flies per culture.

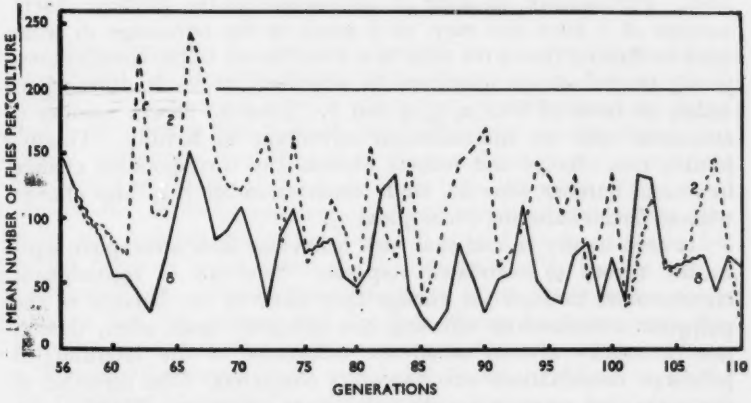

FIG. 8.—Fertility in lines 2 and 8 between S-56 and S-110. Both were selection lines. Line 8 is here shown solid, whereas it was broken in fig. 7. No change in selection is implied by this alteration of style.

latter would never come into being within the mass line itself. The fertility of line 2 suggests that this may have been the situation. A direct comparison with line 7 is of course impossible, owing to the difference in method of keeping the mass and selection lines. But line 2 had at first a lower fertility than the average of line 8, and one

which was indeed itself falling for 5 generations. Thereafter it rose and stayed above the average of line 8 until S–90, a period of some 35 generations (fig. 8). The later change in chaeta number of line 2 was unaccompanied by any noticeable change in fertility for lines 5 and 2 had much the same average fertility.

The conditional nature of the selective disadvantage of the low chaeta combinations is suggested by the low selection from line 3. In this selection the fertility, as we measure it by average number of offspring per culture, rose for the first 5 generations. Thus the impediment, which was sufficient to prevent the low chaeta combinations obtaining a foothold in the mass, was not such as to lower fertility when measured in the cultures of a selection line. The precise difference between the conditions of mass and selection lines can only be conjectured ; but competition among the growing flies in a mass culture must be more severe and involve a wider range of genotypes. There must also be a keener competition among the males for mates (see Bateman, 1948), and among the females for early laying, in a mass than in a selection line.

The response of fertility to selection for chaeta number shows that a relation must exist between these characters, but it is clear from these various findings that relation is not a simple one. A fall in fertility can accompany both increase and decrease in chaeta number under selection, as in the original high and low selection lines. Furthermore, in spite of heterogenicity, the average chaeta number of a mass line may, as a result of the advantage in what must be fertility (using the term in a broad sense) of the combinations giving central chaeta numbers, be stabilised at levels differing as widely as those of lines 2, 3, 4 and 7. Thus no chaeta number is associated with an unconditional advantage in fertility. Finally, fertility may change and recover without any corresponding change in chaeta number (line 8), while chaeta number may also change without fertility altering (lines 5 and 2).

It need hardly be said that such behaviour finds a complete basis in the theory of correlated response. Selection of recombinant chromosomes through the change they show in the balance of the polygenic combinations affecting one character must often, though not necessarily always, mean the unbalance of the intermingled polygenic combinations affecting other characters. The direction of the correlated response in the subordinate character, like its very occurrence, will depend on the detailed organisation along the chromosome of the different polygenic combinations with respect to one another ; but where the subordinate character is one which like fertility depends on a delicate genic balance, the alteration must generally be for the worse. The same process of recombination, if continued sufficiently long, will permit the subordinate character to be readjusted at some new level of the capital one ; but the attainment of full readjustment is likely to be a lengthy process if the

intermingling is complex, so that for a long time any further change of the subordinate character induced by back-selection for the capital one is likely not to be so disastrous as that induced by the first selection.

No simple scheme of pleiotropic action, on the other hand, can account for these findings. They would require four types of genes : (i) those in which the allelomorph associated with raised chaeta number also gives lowered fertility, (ii) those in which the allelomorph associated with lowered chaeta number also gives lowered fertility, (iii) those which affect chaeta number but have no effect on fertility, and (iv) those which have no effect on chaeta number but affect fertility. These four types represent all the possible associations of gene differences affecting chaeta number and fertility taken either singly or jointly. Such an interpretation differs in fact from the theory of correlated response through linkage only in that the latter provides an account of the mechanism by which the various associations come into being, and are changed or lost. If we consider correlated responses in terms of the effective factors, the segments of chromosome which are the units of immediate segregation and response to selection, we can see that these units must be pleiotropic in their action. Theirs, however, is a pleiotropy which, like their action as expressed in any single character, will depend on their internal organisation, and will be capable of being altered and built up by recombination within them.

(f) Delayed responses to selection

Three responses to selection observed in the later stages of the experiment call for special comment. Line 8 responded sharply to selection between S–24, when it was begun by reselection from line 3, and S–28 when it had reached (and indeed for one generation just passed) the general level of 53 or 54 chaetæ. In spite of the continued selection it was stable at this level, apart from temporary fluctuations, up to S–82. At S–82, however, its chaeta number began to rise once more and had become 57 by S–84. It then fluctuated a little, but settled down to hover about the general level of 56 for the rest of the experiment. Comparison with line 7 in the chromosome assays suggests that the increase was due to a change in chromosome III. Selection was thus ineffective for over 50 generations ; it then produced a response for 2 generations ; and finally became ineffective again for the remaining 50 generations over which its action has been observed.

In line 9 the first period of stability was shorter. The chaetæ reached 54 at S–87, rose above this figure for 2 generations and then settled down to fluctuate round it until S–110, in spite of the continued selection. It was during this period that the assay termed 9-1 was made. At S–110 generation a response began which was not completed until S–116. Two of these 6 generations were, however, wasted by the necessity of relaxing selection to cope with the difficulty of low fertility. Apart from a further period of relaxation between

S–121 and S–123 the line has maintained its new level of about 63 chaetæ since S–116. The assay termed 9·2 was made when the line had reached this new level. The chromosome assays show that the increase chaeta number is due, at least mainly, to a change in chromosome III. Though the first period of stability was only about 23 generations as compared with over 50 in line 8, the pattern of behaviour was the same as in that line—a long period of failure to respond to selection, a sharp response, and then stability again.

Line 2 showed similar behaviour under continuous selection for low chaeta number. Begun by back-selection from line 7 at S–56, it had fallen to the middle level of chaeta number by S–65 and fluctuated round 46 or 47 until S–81. A further and larger response began somewhere between S–81 and S–83, the data not permitting a more exact timing of the response (fig. 6). The main fall, which comparison of the chromosome assays of lines 2 and 5 shows to have been due to changes in all chromosomes, lasted until S–90, but there is evidence of a much slower continuation merging insensibly into stability which then lasted until selection was discontinued at S–111. In this line the first period of stability was shorter even than that of line 9, a mere 15 generations or so, and the succeeding fall was longer ; but again we can recognise the same pattern of behaviour.

We can also see this pattern in two of the lines Sismanides (1942) selected for increased number of scutellar bristles. These two lines, C and D, originated from common parents and their remarkable similarity in behaviour can leave little doubt that they were genetically like one another, constituting, one might say, a duplicate experiment. Line C showed a first major response between the second and third generations of selection. In D the response came 4 generations later, but it was both of exactly the same size and due to recombination within the same chromosome (II). After the first response C was stable or almost so, under continued selection, until generation 14 when it once more began to change, reaching a new level and near stability at generation 17. Line D was again a little slower, beginning its second response at generation 16 and continuing until 19. Like the first responses, these second ones were due to changes of the same effect in the same chromosomes (II and III) in both lines.

Here then we have 5 cases where periods of stability, the shortest of which was 9 generations (in Sismanides' line D) and the longest 55 (in our line 8), were followed by marked responses to the same selection that had earlier been ineffective. To what must these delayed responses be attributed ? Certainly not to any change in the mode of selection, for, with rare exceptions not associated with the onset of the delayed rises, this was kept constant throughout the history not merely of each line but of the whole experiment. We must look for the cause on the sudden appearance of variability after a long period when none was available for selective response.

The polygenes affecting chaeta number are known to mutate

(Mather and Wigan, 1942), but there are several obstacles in the way of regarding these delayed responses as due to what is normally implied by point mutation. In the first place the responses which were obtained in Sismanides' two lines, each maintained as an independent breeding unit, depended on changes in the same chromosomes (II and III) and were also of almost the same magnitude. Line C advanced from a mean of about 4·65 scutellar bristles to one of about 5·15. Line D rose from much the same initial value to about 5·25. Admittedly there is a difference of 0·1 in the sizes of these responses, but this is only 20 per cent. of the smaller of them.

The second obstacle is that the responses in Sismanides' lines occurred within two generations of one another in spite of their periods of stability under selection having been 11 and 9 generations respectively. The two lines, and even the two chromosomes within each line, failed to change over much the same lengthy period and then changed within two generations of one another. There is a determinacy about the changes in lines, chromosomes, time and size of total response, which we should hardly expect from point mutation.

The same simultaneity is shown even more strikingly in our own experiments. Line 2 was stable for about 15 generations, but when it did change all chromosomes showed the effect. The change in this line came between S–81 and S–83, while line 8 which was stable for 55 generations also began its change at S–82. The two changed within a generation of one another ; that is to say, since the different generations of the various lines were kept in step, within two weeks of one another. Again we have an appearance of determinacy in time difficult to reconcile with the idea of point mutation.

Finally, the responses in chaeta number were not the only signs of change in lines 2 and 9. After it had stabilised at the lowest level, line 2 was discovered to show a characteristically anomalous pigmentation in all its males. In line 9 the males also showed anomalous pigmentation for the first time after the delayed response, this being a different type of anomaly and one which occurred in only a proportion of the flies. These pigmentary variants will be described more fully in section 4 (*c*) ; it is sufficient for our present purpose to observe that at least in line 9 the evidence points to the altered pigmentation having arisen at the same time as the delayed response occurred in chaeta number. In line 2 the evidence is less clear but would agree with this view. Line 9 also showed a change in the spermathecal content of the females about the time of the delayed response (section 4 (*a*)). All these changes seem to be correlated responses.

The observations all fall into place if the changes are regarded as due, not to point mutation in the strict sense, but to recombination within the chromosomes. In all the lines responses to selection had preceded the first periods of stability, responses which assays show to have depended at least in part on recombination in the very chromosomes to which the delayed responses were later traceable. Thus the

full harvest of response from those recombinations which occur regularly within the chromosomes must have been gathered in these early changes. During the period of stability the lines must therefore have been either homogenic or, more likely, balanced so that heterozygotes were always favoured by selection in regard to those parts of the chromosomes which showed this regular recombination. But we know that there are sizeable portions of the chromosomes, lying near the centromeres, in which crossing-over and recombination are rare. The first response to selection need not have involved any alteration in these regions. We also know that the occurrence of crossing-over within these regions is particularly susceptible to the action of non-heritable, external agencies such as temperature. A chance environmental change, possibly of only temporary occurrence, could therefore bring about a redistribution of crossing-over such as would cause recombination of genes hitherto virtually immune to it. All lines and all chromosomes would be affected simultaneously, but only those would show a release of variability in which the organisation of the polygenic combinations was such that the recombination could be effective in leading to an upset of balance. The occurrence of correlated responses would also, of course, depend on the organisation and intermingling of the polygenic combinations. If two lines, like those of Sismanides, were genetically almost duplicates the results of the changes in distribution of crossing-over, in particular the responses to which they gave rise, must be closely alike. And once the immediate release of variability had been used up in response to selection each line must stabilise again until such time as an even more drastic redistribution of crossing-over was induced by environmental action, for a mere repetition of the first upset must obviously be without effect.

Thus all the special features of the delayed responses to selection, their determinacy in time, in size and in chromosomes responsible, as well as their correlated responses, can be understood if they are referred to new recombination arising from the redistribution of crossing-over which many experiments have shown to follow changes in external conditions. As to what environmental changes could most successfully and most often bring about such release of variability only further and specially designed experiments can show. Such experiments, if successful, would offer a powerful tool for the furtherance of improvement by selection of crops and stock, for they would open the way to the utilisation of hitherto largely untapped reservoirs of variability.

(*To be concluded*)

THE DISTRIBUTION OF POLYGENIC ACTIVITY ON THE X CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

THE theory of polygenic balance supposes the expression of a quantitative character to be under the control of a number, probably a large number, of genes with small similar and supplementary effects. Within an established mating-group the polygenes must be balanced in such a way that the chromosomes coming together in any zygote will generally produce a phenotype approximating to the mode of the population, which will itself approximate to the optimum under the existing environmental conditions. As recombination occurs in every generation new arrangements of polygenes must continually arise; the great majority of these will fit into the existing balance system, but some will not do so, they will be unbalanced and will provide the variation upon which natural selection can act, and the favoured recombinants will be those which tend to maintain the population near the ever-moving optimum point. Evolutionary success depends upon the release of variability at an appropriate rate, a process which itself depends in part upon the linkage relations of the polygenes (Mather, 1941, 1942, 1943).

It is on these grounds important to find out how the polygenes are arranged in relation to the chromosome length and to the frequency of chiasmata along it. Some indications may be drawn from previous work. Payne (1918) on testing a line selected for high number of scutellar bristles located part of the activity on the X chromosome near and perhaps to the right of the *w*' locus. Mather (1941) selecting for abdominal chaeta number in *D. melanogaster* in a cross between *BB* and + found a correlation between *BB* and high chaeta number in F_2 and subsequent selected generations, although the connection had been the reverse in the parental lines. The same author (1942) studied chromosomes I and III from the selected lines more intensively. In the sex chromosomes the extreme left end was not followed and in the remaining portion no undoubted differences of activity were found, but there was strong indication that some of the lines tested differed in the region near *cv*. In the third chromosome differences were found in the *th* region, situated near the centromere. Sismanidis (1942) in lines selected for high number of scutellar bristles found that from a cross using *y w* and *B* stocks the latter was eliminated

in favour of the wild type, whereas both *y w* and the wild type left end persisted, so providing some evidence of activity at the right end of chromosome I.

To summarise ; indications of polygenic activity have been found on the X chromosome at the left end near *w* (Payne) and *cv* (Mather) and also in the region of *B* (Mather, Sismanidis), on the third chromosome near the centromere (Mather), and finally Mather (1944) has demonstrated the activity of the heterochromatic Y, and of the right end of the X.

The demonstration of polygenic activity, like that of all other genetic activity, depends upon finding differences and most of the experiments mentioned above compared only a few chromosomes, or chromosomes derived from only a single original cross, so that the chance of finding differences was less than might otherwise be the case. In the experiments reported here a survey of the X chromosome has been attempted using a greater number of chromosomes of many different origins.

STOCKS AND METHOD

Nine laboratory stocks of wild type *Drosophila melanogaster* were used : Amherst, Ealing, Ockley, Rothamsted, Samarkand, Sutton Bonnington and Wellington which had been kept in mass culture in this laboratory for at least a year, Florida-4, an originally homozygous stock similarly kept, and Oregon, brother-sister mated for over 170 generations. The activity of homologous regions of wild type chromosomes cannot be compared directly, but each can be compared with a standard marked chromosome and so with each other.

The following tester chromosomes were used :—

1. y^{30e} (0·0) ct^{6} (20·0) wy^{40e} (41·9) *B* (57·0).
2L. *B car* (62·5) *B* from tester 1.
2M. *B car B* from unrelated stock.
3. ct^{6} *v* (33·0) g^{2} (44·4).
4. *y. ec* (5·5).

The marked males were back-crossed to $\widehat{XX}$ *f* flies with Oregon autosomes for at least 6 generations before use and were maintained by back-crossing throughout the experiments. For use, the marked males were crossed to *y Hw dl-49* m^{2} g^{4} females, also with Oregon autosomes and the inversion heterozygotes crossed to a single male of the wild stock to be investigated. Daughters without *Hw* were mated in pairs to an excess of Oregon males and allowed to lay for two two-day transfers in half pint milk bottles. The eggs were layed and offspring emerged at a temperature of 25° C. The quantitative character chosen for investigation was the number of sternopleural chaetæ on both sides of the fly.

A female heterozygous for a wild type X chromosome and one

with 4 marker genes will (if multiple recombination is ignored) give 8 types of sons, and one with two markers 4 types. For example *B car*/+ will give the types of sons shown in table 1.

TABLE 1

Recombination in region	Phenotypes	Designation
0	+ +	a
	B car	b
1	+ *car*	c
	B +	d

Using the designation in column 3 table 1. **a** differs from **c** in the region of *car* which, in one is derived from the wild type chromosome, and in the other from the tester, while **d** differs from **b** in the same way; therefore these two pairs of differences each provides an estimate of the relative activity of the chromosome region associated with the *car* locus.

The method of differencing used (Mather, 1942) is given in some detail in tables 2*a* and 2*b*. The sternopleural chaeta number was determined for all classes on a maximum of 10 males from each bottle; if any class was entirely unrepresented the bottle was discarded. In table 2*a* the means of these determinations and the

TABLE 2*a*

Method of differencing

Column 1 Class	Mean bristle number of classes				Differences of class means			
	2 **a** (++)	3 **b** (*B car*)	4 **c** (+ *car*)	5 **d** (*B* +)	6 **a**−**c**	7 Weight	8 **d**−**b**	9 Weight
Bottle 1*a* . .	18·60 10	18·60 10	18·50 4	18·33 3	0·10	2·86	−0·27	2·31
,, 1*b* . .	17·70 10	17·90 10	18·78 9	18·90 10	−1·08	4·74	1·00	5·00
,, 2*a* . .	19·50 10	18·20 10	18·90 10	18·50 6	0·60	5·00	0·30	3·75
,, 2*b* . .	18·40 10	17·60 10	17·29 7	18·29 7	1·11	4·12	0·69	4·12
,, 3*a* . .	19·90 10	19·20 10	17·60 10	17·75 4	2·30	5·00	−1·45	2·86
,, 3*b* . .	19·30 10	18·60 10	19·00 9	17·88 8	0·30	4·74	−0·72	4·44
,, 4*a* . .	18·00 10	18·50 10	18·67 3	17·67 3	−0·67	2·31	−0·83	2·31
,, 4*b* . .	19·20 10	19·00 10	18·67 3	18·13 8	0·53	2·31	−0·87	4·44
Sum of products					15·3486	31·08	−4·7798	29·23
Weighted mean difference					0·49		−0·16	

car region of Amherst chromosome from experiment 5A with tester 2M (*B car*)

numbers of flies on which they were based are given in columns 2 to 5 for four replications each with two transfers; since all comparisons are within the individual bottles environmental factors are reduced to a minimum. Generally, as in this case, 8 bottles were used, but

if any had to be discarded the results were based on the remainder without correction. Columns 6 and 8 give the differences between means in columns 2 and 4 and 5 and 3 respectively. As the means compared are based on varying numbers of individuals up to 10 they must be weighted; an appropriate system is described by Mather (1942) in which the weight used is the product of the two numbers on which the means are based, divided by their sum. In the first entry in column 6 for example, the difference is between a mean of 18·60 based on 10 observations and one of 18·50 based on 4, so that the appropriate weight is $\frac{10\times4}{14} = 2{\cdot}86$, as shown in column 7. Weights similarly obtained for the **d**—**b** differences are given in column 9. To obtain the mean weighted difference, the products of the individual differences and their weights are summed and divided by the sum of the weights. In table 2*b* the weighted mean differences are set out in columns 2 and 3 for the Amherst chromosome (used as the example in table 2*a*) and the four other chromosomes included in the fifth experiment.

As explained above, each of the two differences for any chromosome are independant estimates of the activity of the part of the chromosome under investigation relative to the activity of the tester chromosome, in this case the region round *car*; therefore half their differences (given in column 4 table 2*b*) provides a measure of the variation due to the conditions of the experiment. The total sum of squares of these differences is 0·190075 with 5 degrees of freedom, but one component of this statistic may be isolated as the overall difference between the two estimates. Such a difference would be due to the interaction of loci along the chromosome, and will be found as the square of the sum of the differences, divided by their number—the familiar " correction term " of this type of analysis. Subtracting this correction term gives a corrected sum of squares of 0·069950 with 4 degrees of freedom. The significance of the difference between the estimates in columns 2 and 3 may be assessed by comparison with the corrected mean square, the square root of the variance ratio (2·62) being a **t** with 4 degrees of freedom. In this case it approaches, but does not reach the 5 per cent. level of probability and need not be regarded as significant, so that the uncorrected mean square 0·038015 with 5 degrees of freedom may be used as the estimate of error in subsequent stages of the analysis.

Column 5 is half the sum of the two estimates and its variance measures the differences between the chromosomes tested. The corrected mean square of 0·442718 gives a ratio with the error variance of 11·65, which with N_1 and N_2, 4 and 5 respectively, has a probability between 0·01 and 0·001. The correction figure of column 5 shows the overall difference between the wild type chromosomes and the tester, which is not significant. Finally, inspection of column 5 suggests that the Oregon chromosome differs from all

the rest. To test the significance of this difference the Oregon item was multiplied by 4, the number of other chromosomes in the experiment and the sum of the rest was subtracted from it. The remainder squared and divided by 20 gives a mean square with

TABLE 2*b*

Analysis of variance

Column 1	2 a−c	3 d−b	4 Half of columns 2−3	5 Mean of columns 2+3
Amherst . . .	0·49	−0·16	0·325	0·165
Ealing . . .	0·79	−0·84	0·025	−0·815
Ockley . . .	−0·40	−0·60	0·100	−0·500
Oregon . . .	1·16	0·63	0·265	0·895
Samarkand . .	−0·24	−0·36	0·060	−0·300

	S.S.	D.F.	M.S.	Correction term/ corrected M.S.	$t\ (=\sqrt{\text{V.R.}})$	P
Error variance (column 4)—						
Between chromosomes . .	0·190	5	0·038	...	...	...
Between estimates (correction term)	0·120	1	0·120	6·87	2·62	0·10-0·05
Corrected S.S. . . .	0·070	4	0·017	...	...	...
All chromosomes (column 5)—				V.R.		
Between chromosomes . .	1·832	5	...	...	...	...
Between tester and chromosomes (correction term)	0·062	1	0·062	1·62	1·27	0·30-0·20
Variation between chromosomes	1·771	4	0·443	11·65	...	0·01-0·001
All chromosomes without Oregon—						
Between chromosomes . .	1·031	4	...	...	...	...
Between tester and chromosomes (correction term)	0·526	1	0·526	13·83	3·72	0·02-0·01
Variation between chromosomes	0·506	3	0·169	4·44	...	0·20-0·05
Difference of Oregon and rest— $\left[\frac{(4\times 0\cdot 895)-(-1\cdot 45)}{20}\right]^2$	1·265	1	1·265	33·28	5·77	0·01-0·001
Internal error variance . .	0·190	5	0·038	...	...	...

one degree of freedom which is significant. The analysis of the remaining 4 chromosomes was carried out in the usual way and shows that in fact the significance of the variance of the 5 chromosomes was due almost entirely to the Oregon item which differs very significantly from the other four, which do not in this analysis differ significantly among themselves.

In this example the variances are compared with the error variance derived from the same experiment, but for convenience and precision it is desirable to pool the error variances of all the experiments. Not all comparisons are independent of each other,

for instance, the determination of the *car* region is based on comparisons between wild type and *car* and *B* and *B car* while that of the *B* region uses the same figures in comparing wild type with *B* and *car* with *B car*. One group was therefore made of the error variances from the left-most region in any test and any others independant of it (that is *y* and *wy* from tests with *y ct wy B* and *B* from those with *B car*) and another from the right-most region (*ct* and *B* from tester 1 and *car* from tester 2) and the error variance from the *y* region with tester 4 was included in the left-hand and that from the *v* region with tester 3 in the right-hand group. As the bias shown by the correction figures was not significant the uncorrected error mean squares were used throughout. The two groups given in table 3 were tested for hetero-

TABLE 3

Homogeneity of error variances from separate tests

Experiment	Region	S.S.	D.F.	Experiment	Region	S.S.	D.F.
1	*y*	0·656625	9	1	*ct*	0·685700	9
1	*wy*	0·416125	9	1	*B*	0·188950	9
2	*y*	0·302675	9	2	*ct*	0·631450	9
2	*wy*	1·219375	9	2	*B*	1·015050	9
3L	*B*	0·103200	5	3L	*car*	0·183500	5
3M	*B*	0·063475	5	3M	*car*	0·063450	5
4	*B*	0·070500	3	4	*car*	0·073275	3
5A	*B*	0·195050	5	5A	*car*	0·190075	5
B	*B*	0·257975	5	B	*car*	0·322975	5
C	*B*	0·447575	5	C	*car*	0·449275	5
D	*B*	0·243875	5	D	*car*	0·222525	5
7	*y*	0·057979	6	6	*v*	0·405100	6
Overall M.S. . .		0·053792	75	Overall M.S. . .		0·059084	75
Test for homogeneity $\frac{0·105709}{0·005788} = \chi^2_{(11)}\ 18·26$ P 0·10—0·05				Test for homogeneity $\frac{0·068223}{0·006982} = \chi^2_{(11)}\ 9·78$ P 0·70—0·50			

geneity by the formula due to W. L. Stevens (Fabergé, 1936). The pooled error variance from the second group 0·059084 being the larger and more homogeneous was used throughout in all tests of significance, so that N_2 is always 75. Where the larger variance has only one degree of freedom the square root of the ratio is treated as a normal deviate.

RESULTS.—THE RIGHT END

In the first experiment 9 wild type chromosomes were tested with *y ct wy B*. In table 4 the analysis of the sums of the two estimates of the effects of the right end in the region round *B* based on comparisons between wild type and *B* and *y ct wy* and *y ct wy B* is given. Although when all the chromosomes are considered they show a significant

difference, this on further investigation is found to consist solely of a difference between Oregon and all the rest, which do not differ significantly amongst themselves. There is also a highly significant difference between the tester and all chromosomes except Oregon. Since this experiment had tested X chromosomes which had been back-crossed to $\widehat{XX}f$ with Oregon autosomes for at least 12 generations, it was repeated, using chromosomes straight from the stock bottles: the results of this second experiment were very similar and are given in the last three columns of table 4. Therefore the lack of variation

TABLE 4

B *region from tests with* y ct wy B

	Experiment 1				Experiment 2		
	D.F.	M.S.	V.R. or *t*	P	M.S.	V.R. or *t*	P
Between all chromosomes . .	8	0·205	3·47	0·01-0·001	0·227	3·85	0·01-0·001
Between Oregon and rest . .	1	1·413	4·89	<0·001	1·243	4·59	<0·001
Between rest without Oregon .	7	0·033	...	...	0·082	1·39	>0·20
Difference between rest and tester	1	13·847	highly	significant	11·186	highly	significant
Pooled error variance . .	75	0·059	...		...	...	

The larger overall M.S. from table 3 is used as error variance in this and all later tables

in the region round *B* cannot be ascribed to any possible effect of its association with the Y chromosome from the $\widehat{XX}$ stock.

In order to investigate the right end of the chromosome further, two *B car* testers were made up. In one (2L) the *B* locus came from tester 1 and in the other (2M) from a supposedly unrelated stock. The locus of *car* came from the same source in both testers. A single male from each of 5 stocks (including Oregon) was tested with both these testers in experiments 3L and 3M. In these tests the chromosome is divided into two, for the estimation of polygenic variability; the proximal part, extending from a point midway between *B* and *car* includes the main heterochromatic region of the chromosome and is marked by *car*, and the essentially euchromatic distal part is marked by *B*. In view of the extensive recombination to the left of *B* the data for this region are not critical; the right end is, however, well marked by *car*. The results of the analysis are set out in the top two lines of table 5; both testers give very similar results, except for their difference from the 4 chromosomes without Oregon (last three columns). This difference is clearly due to sampling error, as tester 2M was used in all subsequent experiments, in which it shows a significant difference from these chromosomes.

Experiment 4 tested the two stocks (with Oregon) which showed the maximum range of variation in experiment 3M; this variation was not recovered, presumably because the two stocks were not inbred.

To investigate the relation of X and Y chromosomes experiment 5 was set up. Males from 5 stocks (including Oregon) were tested with 2M and the identical chromosomes were kept for 3 generations against the same back-cross stock of $\widehat{XX}$ females used to keep the tester and

TABLE 5

car *region*. *All tests with* B car *testers*

Experiment	All chromosomes				Without Oregon			
	D.F.	M.S.	V.R.	P	D.F.	M.S.	V.R.	P
3L	4	0·349	5·90	<0·001	3	0·008	...	...
3M	4	0·266	4·50	0·01-0·001	3	0·092	1·55	0·20
4	2	0·214	3·62	0·05-0·01	1	0·001	...	...
5A	4	0·443	7·49	<0·001	3	0·169	2·85	0·05-0·01
B	4	0·255	4·32	0·01-0·001	3	0·013	...	...
C	4	0·121	2·04	0·20-0·05	3	0·056	...	...
D	4	0·534	9·04	<0·001	3	0·210	3·55	0·05-0·01
Sum of 5A-D	...	...	...	...	12	0·112	1·89	0·05
Part of 3 and 5	...	...	...	...	12	0·103	1·73	0·20-0·05

Experiment	Oregon item			Difference of chromosomes (without Oregon) from tester		
	M.S.	*t*	P	M.S.	*t*	P
3L	1·370	4·82	<0·001	1·227	4·56	<0·001
3M	0·788	3·65	<0·001	0·051	...	...
4	0·427	2·69	0·01-0·001	0·120	1·42	0·20-0·10
5A	1·265	4·63	<0·001	0·526	2·98	0·01-0·001
B	0·981	4·08	<0·001	0·526	2·98	0·01-0·001
C	0·315	2·31	0·05-0·02	0·893	3·89	<0·001
D	1·507	5·05	<0·001	0·570	3·11	0·01-0·001

re-tested in each generation. Any influence of the Y in this region would tend to make the wild type chromosomes more like the tester and would be reflected in a decrease in significance over the last four entries in the final columns in table 5. Clearly no such decrease occurs in the course of 3 generations, but this is not surprising as Mather (1944) found considerable variation after 5 generations of crossing to $\widehat{XX}$, and its subsequent decay was irregular.

Since the 4 chromosomes remained unchanged throughout experiments 5*a* to 5*d* the results may be summed. This is done in the one from last line of table 5 and the variance ratio just attains the 5 per cent. level of probability (1·89 with 12D.F.). A further test with 12 degrees of freedom could also be made, because chromosomes from the Amherst, Ockley and Samarkand stocks were tested both in experiment 3 and 5. Although the chromosomes tested in experiment 3 may have differed from those tested in experiment 5*a* to 5*d* (as the

stocks from which they came were mass-cultured) any difference there might have been between experiments was not significant and the mean squares for the 3 chromosomes in all 6 tests may properly be summed (last line of table 5), the ratio approaching the 5 per cent. level of probability.

The consistently significant difference between the Oregon chromosome, and the testers on the one hand, and the other 8 chromosomes tested on the other, shows the right end of the chromosome may greatly affect sternopleural bristle number. The thorough analysis of the fifth experiment (which contains the greatest amount of data) corroborated by the analysis of the three stocks tested in the third and fifth experiments shows that even over and above this rather clear cut difference there are indications of further variation in this region.

CENTRAL PORTION

The *y ct wy B* tester used in the first two experiments gave estimates for the two regions centering on *ct* and *wy* and for the region embracing both these loci. The analysis is shown in table 6. The great increase in significance of the two regions taken together suggested that there was either a centre of variation about half-way between the two loci, or, if the two loci were each associated with a separate centre of activity, these must be acting in the same direction. The possibility of a single centre of variation was tested in experiment 6 using a *ct v g* chromosome as tester. Only the *v* comparison is reliable with this tester, and it failed to reveal any significant difference in this region, as shown in the bottom line of table 6. There must then be

TABLE 6

Central region

	ct region				*wy* region				*ct wy* region			
	D.F.	M.S.	V.R.	P	D.F.	M.S.	V.R.	P	D.F.	M.S.	V.R.	P
Between chromosomes experiment 1	8	0·130	2·19	0·05-0·01	8	0·084	1·42	>0·20	8	0·246	4·17	0·001
Between chromosomes experiment 2	8	0·228	3·85	0·01-0·001	8	0·170	2·88	0·01-0·001	8	0·350	5·92	0·001
									v region			
Between chromosomes experiment 6									5	0·057	...	...

two centres of activity, near *ct* and *wy* respectively, which act in the same direction in the 9 chromosomes tested, although the action is in a plus direction in some chromosomes and in a minus direction in others. Furthermore these centres of activity are divided by a region having no polygenic activity on sternopleural bristle number.

THE LEFT END

The analysis of the *y* region in the first two experiments is given in table 7. In both experiments the variance ratio is highly significant and this region is thus clearly very active in affecting the bristle number, though the average difference is little if any greater than the difference found at the right end, between the Oregon and tester chromosomes on one hand and the other chromosomes tested on the other. In order to localise the active region more exactly experiment 7 was set up using 6 chromosomes with tester 4 (*y ec*). The region marked by *ec* was rejected for similar reasons to those which led to rejection of the *B* data with *B car* testers. The results for the *y* region are given in the last line of table 7 and show the great activity of the

TABLE 7

y region

Between chromosomes experiment 1 .	8	1·005	17·02	<0·001
,, ,, ,, 2 .	8	0·968	16·38	<0·001
,, ,, ,, 7 .	5	1·968	33·30	<0·001

region from *y* to midway to *ec* (0·0-2·8). This result does not preclude the existence of variation also in the remainder of the region covered in experiments 1 and 2 (from 2-8—10·0) ; indeed the variance in experiment 7, being nearly twice that in the other two experiments, might suggest some activity in this region, having an opposite sign to that in the distal part.

DISCUSSION

From the results of these experiments a rough map of the distribution of polygenes controlling sternopleural bristle number begins to emerge, for the X chromosome. Maximum activity is in the left end from 0·0-2·8 on the genetical map ; in the middle part two centres associated with *ct* and *wy* show significant activity and are divided by an inactive region, and the right end from between *B* and *car* to the centromere shows a great difference between the Oregon chromosome and the rest and, in addition, some differences between the chromosomes other than Oregon. This activity is probably underestimated in these experiments since Mather (1944), using a different technique, has shown the existence of considerable activity in the pairing segment of the X and Y and the transference of genetic material between them. Failure to detect it in the present experiments may be connected with the exceptional behaviour of the Oregon chromosome found by Mather in this region, since the right ends of testers 1 and 2L and 2M may all have been ultimately derived from the Oregon stock which is generally used for outcrossing and extraction in this laboratory. In this connection it may be noted that

the difference of Oregon from the other chromosomes is of about the same order of magnitude as the largest difference reported by Mather between the Y chromosomes which he tested.

In considering the distribution of polygenes it must be remembered that in these experiments only one character has been followed. Although the observation of correlated responses (Wigan and Mather, 1942) shows that polygenes controlling different characters may occupy the same chromosome regions, different optimum rates for the release of variability in different characters would disfavour the existence of variability of all characters in all centres of activity. It must also be remembered that these results were obtained on the X chromosome; *Drosophila melanogaster* shows relational balance (Wigan, 1944) and the polygenic organisation of the X chromosome, which is hemizygous in one sex, and must therefore also show internal balance, may not represent the condition in the autosomes, where no such compromise is called for.

For the polygenes controlling sternopleural bristle number the distribution is not continuous, the two centres of activity associated with the loci *ct* and *wy* being separated by an inactive region round *v*. Perhaps a more minute investigation of the chromosome would reveal further subdivisions of the polygenic distribution within the rather large blocks so far tested, and indeed the result of experiment 7 suggests this to be so in the longer region associated with *y* in experiments 1 and 2. It is interesting that the whole of this long region extending from half-way between *y* and *ct* to half-way between *wy* and *B*, a total length of 39·5 units (that is more than half the cross-over length of the chromosome) should be so organised that the two active regions recognised in it have effects in the same direction. If such an arrangement should prove to be general for this region of the X it suggests that the distribution of polygenic complexes having a plus or minus effect is governed not only by the distribution of chiasma frequency and the general requirements of balance but by other undetermined factors as well.

In fig. 1 the cross-over is compared with the salivary map and with the heterochromatin in the metaphase chromosome. The left end of the chromosome, which has great polygenic variability, is also known to contain a high proportion (relative both to its genetic and salivary length) of the recorded visible mutant loci and of chemically induced lethals (Slizynska and Slizynski, 1947); major genes and polygenes are not therefore antithetical in their distribution. Neither are they fully correlated, for the region round *v* shows more major genic loci but less polygenic variation than does the portion of the chromosome marked in these experiments by *car*. Polygenic variation may thus be associated with regions rich in major genes but it may also occur where they are absent.

It will be seen from the diagram that the polygenic activity of the regions tested is not directly proportional to their euchromatic length.

It is also clear that activity is not proportional to the amount of heterochromatin which has also been shown by Mather (1944), who found that females with an extra Y (which is almost entirely heterochromatic) did not differ significantly from their normal sisters.

In addition to the heterochromatic block at the proximal end there are probably many intercalary segments of heterochromatin within the euchromatic portion; the evidence for this, with maps, is given by Slizynski (1945) and Slizynska and Slizynski (1947). Of the first 20 divisions of the salivary map, covering the whole X chromosome (Bridges and Brehme, 1944) only 4 divisions are not

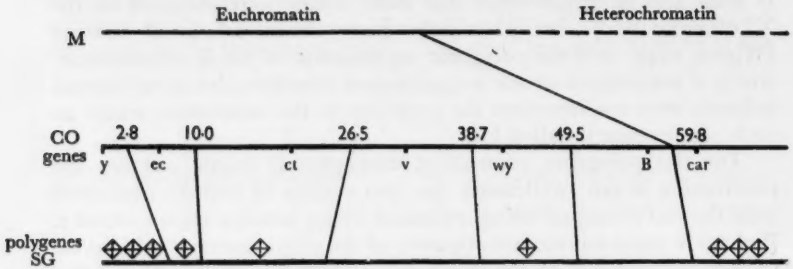

FIG. 1.—Relation of polygenic activity to length of eu- and heterochromatin. Metaphase chromosome above (after Dobzhansky, 1936). Crossing-over map centre. Salivary chromosome below (points estimated after Bridges and Brehme, 1944).

The lozenges indicate roughly the polygenic activity associated with the chromosome regions. At the extreme right end the differences were chiefly between Oregon and the other chromosomes tested, but lesser differences were also found between these latter.

reported to contain some heterochromatin. A single division of the salivary map being, on the average for the whole X chromosome, equivalent to about 3 genetical units of recombination, the regions tested probably each included several heterochromatic segments. Moreover, it has been shown (Prokofieva-Belgovskaja, 1947) that eu- and heterochromatin are not invariable in their limits even in the salivary nuclei of the same individual of *D. melanogaster*, a condition that has also been reported in *D. nebulosa* (Pavan, 1946) and *D. pallidipennis* (Dobzhansky, 1944), while Kosswig and Shengun (1947) show these limits to be variable in *Chironomus* in polytene nuclei in different tissues. It is not therefore possible from these results, to establish any causal connection between polygenic action and the presence of intercalary heterochromatin.

SUMMARY

The distribution of polygenic activity affecting the number of sternopleural bristles in *D. melanogaster* was examined in 9 X chromosomes from laboratory wild stocks.

Maximum activity was found at the left end (0·0-2·8); the regions round *ct* (10-31) and *wy* (31-49·5) both showed significant activity

which tended in the same direction in any one chromosome; no activity was found near *v* (26·5-38·7). At the right end, in the region round *car* (59·7-centromere), there was a highly significant difference between the inbred Oregon chromosome and the testers on the one hand and the other 8 chromosomes tested on the other; the other differences found in this region were significant just at the 5 per cent. level.

Polygenic activity was shown not to be directly proportional to cross-over length or to the amount of euchromatin shown on the salivary map; nor was it proportional to the amount of heterochromatin in the metaphase chromosome. Owing to the general distribution of intercalary heterochromatin along the X chromosome no connection could be established between polygenic activity and heterochromatin.

In the chromosomes tested polygenic activity was not fully correlated with the distribution of major genic loci, but the greatest polygenic activity was associated with a region having a high concentration of visible and lethal mutations.

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THE QUANTITATIVE STUDY OF POPULATIONS IN THE LEPIDOPTERA

2. *MANIOLA JURTINA* L.

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1. Introduction
2. Material
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4. Interchange between colonies, and variability
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1. INTRODUCTION

On a previous occasion (1), the present authors showed that an isolated population of Lepidoptera (*Polyommatus icarus*, Rott.) provided particularly suitable material for quantitative study, and that by adopting a system of marking and releasing, the numerical density could be calculated from the proportion of marked specimens subsequently recaptured. Moreover, it is possible to construct a life-table for the insects comprising such a population, and to determine whether it agrees with any given daily elimination-rate, (2). This may be a constant one, or the proportion eliminated may differ from one age-group to another.

Now it frequently happens, as we in fact found, that a community becomes subdivided into a number of smaller colonies by minor ecological barriers. In such circumstances, it is clearly desirable to determine quantitatively the extent to which *leakage* occurs between one inhabited area and the next. This aspect of animal movement is one which tends frequently to be overlooked, although it represents the normal condition found in most communities. In contrast, the occurrence of *migration*, to which so much study has been devoted, must be regarded as an abnormal and special phase to which, even in the most migratory animals, only a small part of their total life is committed. The method of marking by dots of cellulose paint,

differing in colour and position, which we have adopted is well fitted to detect such leakage from one to another neighbouring colony. For the individuals in each may be given distinguishing marks in addition to those used to indicate dates of capture.

These various lines of approach open up a possibility of considerable ecological and evolutionary interest. That of measuring and comparing survival-rate in different colonies of known size, between which the amount of interchange can be determined.

With this object in view, two of us (W.H.D. and E.B.F.) spent the period 14th August to 3rd September 1946 on the uninhabited island of Tean, Isles of Scilly. We wish to express our thanks to Major A. A. Dorrien-Smith for his kind permission to camp there.

2. MATERIAL

Our previous experience on Tean in 1938 led us to select the butterfly *Maniola jurtina* L. as suitable material for this study, and the choice proved to be justified. It is abundant on the island so that we were able to capture adequate samples without difficulty. Indeed, it seldom took the two of us more than an hour and a half to collect our full daily quota of 120 specimens. Furthermore, it is large enough to be handled easily during the work of marking and recording.

Perhaps its greatest advantage from our point of view is its willingness to fly in almost any kind of weather. At temperatures of 60° F. and above, it is active even in the rain and without sunshine. Below this temperature it tends to become sluggish and requires a good deal of rousing. Only a strong wind combined with a low temperature rendered collecting impossible. This characteristic was a great asset not only in obtaining our sample but also because of the speed with which marked specimens redistributed themselves within the population when released. Thus we found that two hours of sunshine or even of warm dull weather were quite sufficient for randomisation to occur. Another valuable quality of this species is its freedom from large-scale migration, a feature which we tested experimentally (see section 4). Moreover, it tends to become restricted to certain well defined areas within its range. We only found it where the grass was long, and this was limited to places where gorse, bracken, and bramble protected it from grazing by a few cattle generally kept on the island and from strong winds and salt spray. Elsewhere there was a growth of short turf unsuitable for colonisation by *M. jurtina*.

3. LOCALITY

In our previous study of *Polyommatus icarus* (*l.c.*) we found that the island of Tean could be divided into a number of distinct regions. Those over which we collected were populated by the butterfly while

the remainder were either shut off by effective barriers or covered by such vegetation as to prevent colonisation by that species. As already indicated, the distribution of *M. jurtina* is similarly localised but, owing to its different habits, the barriers and habitats are approximately reversed. Thus two areas in which we had previously met with *P. icarus* abundantly proved to be formidable barriers to *M. jurtina*.

We found that the island could conveniently be divided into five regions (see fig. 1, map). Of these, 1, 3, amd 5 are colonised by *M. jurtina* while 2 and 4 are not. In each of the three collecting areas, we chose a central point at which all the insects captured in it were released after marking. Exceptions to this procedure were instances

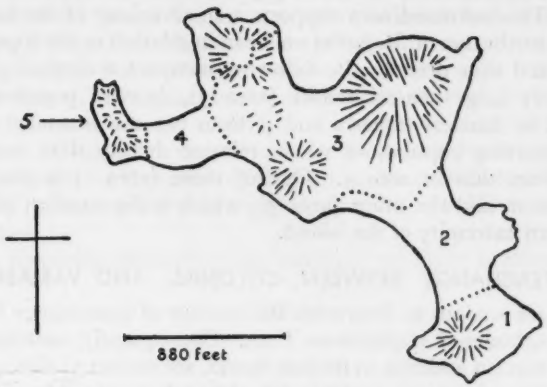


FIG. 1.—Tean (Isles of Scilly). Boundaries of collecting areas .....

in which butterflies had strayed from one area and been recaptured in another (see section 4). On such occasions they were liberated, after appropriate marking, in the area from which they had originally come.

Area 1 comprises about one third of the southern limb of the island. At its extremity is a small hill, about 50 feet high, most of which is covered with a dense growth of bracken, bramble, and gorse. Interspersed with this are patches of long grass of various species, which are the favourite habitats of *M. jurtina*. Near the shore, and also to the north, the ground becomes more wind-swept, the bracken diminishes, while bramble and gorse disappear altogether. Associated with this change is a reduction in the quantity of long grass and hence in the number of *M. jurtina*. Area 2, which comprises the northern part of the southern limb of the island, is completely wind-swept and covered only with short stunted grass and a number of the hardier seaside plants such as *Armeria maritima* and *Erythraea centaurium*. It is not colonised by *M. jurtina* but forms an effective

barrier between areas 1 and 3. Area 3 comprises the main body of the island and includes three hills, of which that to the north east is the highest (about 120 feet). For the most part it is covered with a dense growth of bracken, bramble, and long grass. On the slopes, however, bracken and bramble tend to be replaced by gorse, while on the summits there is little but stunted grass and a carpet of *Calluna vulgaris* and *Erica cinerea*. On the whole, area 3 is densely populated by *M. jurtina*. Area 4 is very similar in appearance to area 2 and includes the greater part of the western limb of the island, thus forming a barrier between areas 3 and 5. Area 5 consists of a small promontory at the extremity of this western limb. This is formed by a mound about 30 feet high on whose eastern side are several patches of bracken and long grass, also a few stunted bramble bushes. This restricted area supports a small colony of the butterfly.

The distribution of *M. jurtina* on Tean in relation to the topography of the island thus presents the following compact ecological picture: a relatively large central massif (area 3), heavily populated and separated by barriers (areas 2 and 4) from two much smaller regions both supporting populations whose relative density does not differ greatly from that in area 3. One of these (area 1) is situated at the southern, and the other (area 5), which is the smallest of all, at the western extremity of the island.

4. INTERCHANGE BETWEEN COLONIES, AND VARIABILITY

We were anxious to determine the amount of interchange between the three colonies of *M. jurtina* on Tean. Consequently, each specimen captured was (in addition to its date-marks, see section 5) distinguished so as to show the area in which it had first been caught. The base of the left hind wing on the underside was marked with a dot of white cellulose paint for area 3 and with a dot of blue for area 5 while it was left unmarked if the specimen had been taken in area 1 (that such an individual was a recapture was of course shown by a dot of paint on some other part of the wings, indicating the date on which it had been taken previously).

During our stay on Tean we recaught in all 183 specimens (99 females, 84 males) which we had previously marked, counting multiple recaptures of the same individual separately. That is to say: 68 (34 females, 34 males) in area 1, 29 (14 females, 15 males) in area 3, and 86 (51 females, 35 males) in area 5. Of this total of 183, only three were recaptured in an area difierent from that in which they had previously been taken, together with an additional specimen (not included in the totals) which was found flying in the middle of a barrier (area 4), half way between areas 3 and 5. The details of these four specimens are given in table 1.

It appears therefore that the amount of interchange from one colony to another on Tean was small and that the minor ecological barriers presented by areas 2 and 4 are rather effective.

Consequently it seemed worth while to measure some variable character which could be used in comparing the populations from

TABLE 1

Specimens of M. jurtina *caught in one area and recaptured in another*

Previous captures	Original area	Recapture	New area	Sex
18, viii	1	23, viii	3	Female
20	5	23	3	Male
24	3	25	5	Female
25, 27, viii	3	1, ix	4	Female

our three areas in order to test whether isolation had caused them to evolve differently. We were handicapped in our choice since we were limited to features which could be studied on a living insect without risk of damaging it. We therefore decided to record the number of spots on the underside of the hind wings, scoring also the sexes. These spots vary from 0 to 5 on each hind wing, and it may reasonably be thought that they are under multifactorial control : a type of variation likely to respond relatively quickly to the effects of selection.

The numerical data which we obtained are given in table 2.

TABLE 2

Numbers of spots per hind wing of Maniola jurtina *from three colonies on Tean, 1946*

Spot numbers	Males				Females			
	Areas			Totals	Areas			Totals
	I	III	V		I	III	V	
0	10	11	2	23	63	82	25	170
1	34	47	6	87	52	73	29	154
2	115	203	21	339	92	145	39	276
3	41	45	7	93	34	55	12	101
4	11	12	2	25	10	10	4	24
5	...	2	...	2	3	1	...	4
Totals . .	211	320	38	569	254	366	109	729

They do not indicate that the three populations differ in the number of these spots. Either isolation had not caused the populations to diverge in this respect or else the numbers were too small to detect the change. However, there is a remarkable and fairly consistent difference between the distribution of these spots in the two sexes,

to which attention should be drawn (fig. 2). It will be observed that each sex has a mode at two spots, but that, while the distribution is approximately symmetrical in the male, it is of an unusual kind in the female. For the higher numbers of spots the fall in frequency is approximately similar in the two sexes, but for the lower the female distribution becomes bimodal. Relatively more females than males have one spot, and this difference is so much increased for those which are spotless that such females are actually more numerous than are those in the one-spotted class. It looks as if more than one

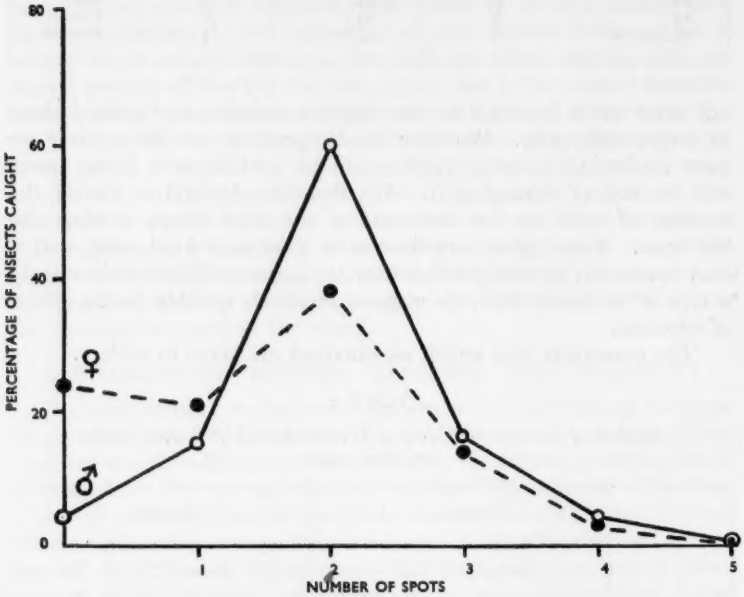


FIG. 2.

genetic type were responsible for the production of spotting in females but not in males : it may be that additional genes, sex-controlled in effect, inhibit spotting in the females.

The result is that, judged on these spot numbers, the female is the more variable sex. It is noteworthy that we have before obtained evidence of this tendency, though without indication of bimodality, in a survey of variability in ground-colour in 35 species of night-flying moths which two of us undertook in 1928 (3). In that sample, we found that the mean variance of the females is about 30 per cent. greater than that of the males. We showed that sex-linkage could not be responsible for this effect, but it has never been satisfactorily explained and deserves further investigation. The occurrence of greater female variability in 35 species of moths, and its reappear-

ance in a somewhat different form in the present butterfly data, suggests that this may be a widespread phenomenon in the Lepidoptera.

5. MIGRATION

We saw numbers of butterflies crossing the sea from one island to another of the Scilly group but never observed *M. jurtina* doing so, and indeed there is no evidence that this is a migratory insect. However, we made a small-scale test to examine the matter somewhat further by experimental means.

On 26th August we captured 46 specimens of *M. jurtina* on that part of the neighbouring island of St Martins where it approaches nearest to Tean. The intervening distance is here only about 300 yards, but the coasts fall sharply away from one another in either direction from that point. We gave these insects a distinguishing mark with red cellulose paint and liberated them again. This sample included none that had been taken on Tean, though we had during the previous ten days released 1003 marked specimens (omitting recaptures) there. Nor were any bearing the St Martins mark taken later on Tean among the 322 specimens (also omitting recaptures) which we caught during the remainder of our stay. On the whole it seems probable therefore that the migration of *M. jurtina* from one island to another is a rare event.

6. NUMERICAL ANALYSIS OF THE THREE *M. JURTINA* POPULATIONS ON TEAN

As already explained, we estimated the numbers of *M. jurtina* in each population on Tean by marking the specimens with dots of cellulose paint. These are permanent, for the paint is water-proof, and it dries in about ten seconds and seals the scales on to the wings. The position and colour of each spot indicates a date of capture. The specimens were put into separate boxes when caught and taken to one of our tents for marking and recording when the samples had been collected. They were subsequently set free at a releasing point in the centre of the area in which they had been captured.

The sizes of the daily samples which we aimed at collecting were 40 from area 1, 60 from area 3, and 20 from area 5. As already explained, areas 2 and 4 constitute barriers and were not populated by *M. jurtina*.

The insects were allowed at least two hours of active flight before a second sample was taken on the following day. Specimens belonging to the most recent sample were constantly found scattered in all directions from the releasing point up to the limits of each colony, for the habits of this butterfly make its distribution largely independent of the direction of the wind. The individuals tend to fly low across or against the wind, and at intervals to drift down with it at a slightly greater height. Moreover, we were careful to randomise our captures,

taking them from all parts of each colony, and we did not find an undue proportion of those recently marked near the releasing points.

It was, however, necessary to guard against the possibility that specimens had been damaged in the marking and were unable to fly or to distribute themselves when released. For this purpose we decided to employ similar precautions to those adopted by Fisher and Ford (1947) in the work on *Panaxia dominula*. Any insects which had obviously been damaged were killed and recorded as such. The remainder were thrown high into the air when liberated. Those which fell without flying were examined and if they appeared undamaged were again thrown into the air. If they fell once more they too were killed and so entered in the records. An area of 10 yards round each releasing point was not collected. This was inspected each day so that any specimens found within it could again be thrown into the air and then killed if they appeared unable to fly. However, *M. jurtina* proved particularly easy to handle without injury, and in fact we only damaged four specimens out of the 1569 which we caught.

The following is an analysis of our numerical data.

The abundance of the data available for *M. jurtina*, the frequency of the days of capture and release, and the distinction of the sexes of all insects captured allow of an examination of the course of change in the population in some respects more detailed than has been attempted on previous occasions.

The method of analysis is that set out in detail by Fisher and Ford (1947) in analysing several years' records of the moth *Panaxia dominula*, and need only be indicated summarily. From the known numbers marked and released on previous days, the number of marks on insects now probably alive and flying in the population available for sampling may be calculated, if the rate of daily elimination is known. An objective estimation of this rate of elimination is obtainable from the number of days between marking and recapture observed in the case of recaptures. Corresponding with any assumed rate of elimination we can, in fact, by direct arithmetic, make an estimate of the total number of days for all marks recaptured, and compare this with what has been observed.

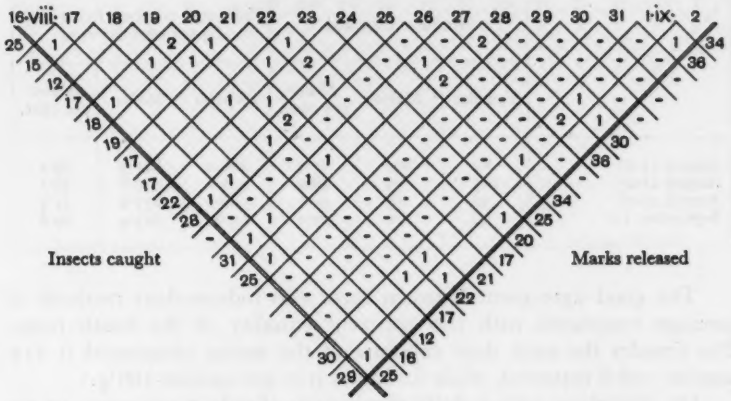
Preliminary trials with the *jurtina* data indicated that in most cases a daily rate of elimination of 11 per cent., corresponding with an expectation of life of eight days, resulted in a satisfactory agreement. Elimination was, however, certainly at a higher rate than this in the small region of area 5, though the similar indication, for the females only, in area 3 is not trustworthy owing to the small numbers caught.

Before considering the special cases it will, however, be clearer if the results of using a single death rate be set out for all areas, and for the two sexes separately. The sex ratio has evidently changed greatly during the course of the sampling, and as it also shows considerable differences between different areas, the treatment of the

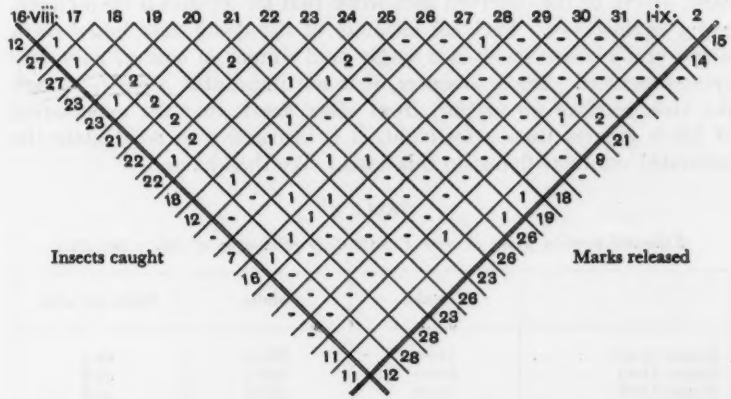
females and males of each area as though they were two distinct populations is in this material particularly important.

The simplest record is that provided by area 1. With independent estimates of the numbers flying both of females and males it is possible to compare the values of the sex ratio at different dates, obtained

M. furtina, 1946, Area 1. ♀.



M. furtina, 1946. Area 1. ♂.



by two distinct methods: (i) from the numbers of insects of the two sexes caught on these dates, (ii) from the numbers estimated to be flying at these dates.

For area 1, the number of marks estimated to be on females at large in the population had been built up to over 50 by the fourth day, 19th August. It reached its peak at 116 on 28th August. For males it was over 50 by 18th August, and was at its highest with

114 on 25th August. Through nearly the whole of the season, therefore, the population had been adequately marked, and each sample of 40 taken daily gave a good basis for estimating the numbers.

TABLE 3

Numbers and sex ratio at different dates in area 1

	Numbers captured			Estimated numbers flying with 89 per cent. survival		
	Females	Males	Males per cent.	Females	Males	Males per cent.
August 17-20 . .	62	100	61·7	466·2	801·3	63·2
August 21-25 . .	103	95	48·0	590·0	546·6	48·1
August 27-28 . .	56	23	29·1	596·0	277·2	31·7
September 1-2 . .	59	22	27·2	530·1	225·4	29·8

The good agreement between these two independent methods is perhaps connected with the apparent equality of the death rates. For females the total days exposure of the marks recaptured is 213 against 196·8 expected, while for males it is 200 against 186·4.

On reworking with a daily elimination of only 7 per cent., or an expectation of life of 13·3 days, the expectation for males is quite close, 198·0, to the observed 200, while that for females is even closer, 213·3 against 213. Such an estimate of the death rate based on a single year's observation in a single area cannot of course, have any great precision. Since, however, it is not impossible *a priori*, though the view receives no support from other areas, that the expectation of life is greater than a fortnight, it is instructive to recalculate the estimated numbers flying on this basis. We then have

TABLE 4

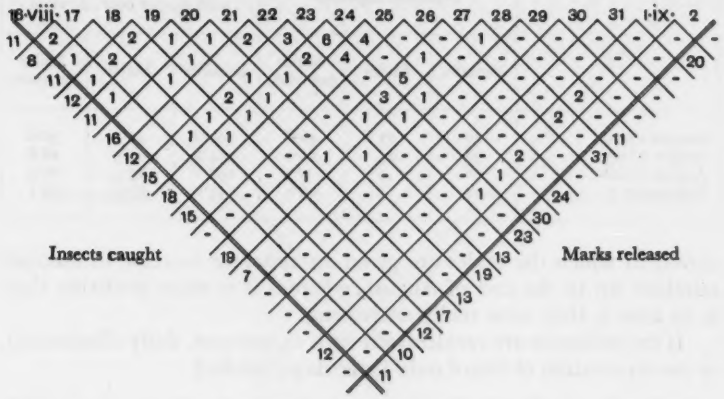
Estimated numbers flying in area 1, with daily elimination of only 7 per cent.

	Females	Males	Males per cent.
August 17-20 . .	510·6	866·2	62·9
August 21-25 . .	697·6	638·5	47·8
August 27-28 . .	751·0	367·3	32·8
September 1-2 . .	746·3	343·6	31·5

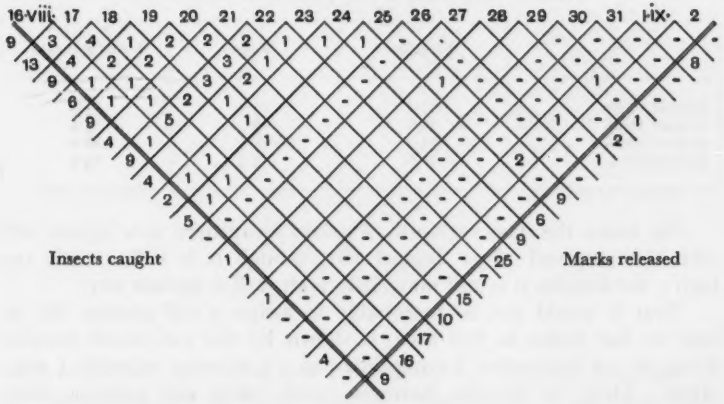
The estimated numbers are appreciably greater, especially towards the end of the season : but the sex ratio is scarcely changed. Males are falling in numbers for the whole period, while the maximum abundance of females is near to the end of August. The total population in the area probably never falls outside the limits 750-1400.

Area 5, with a very small population, of the order of 100 compared with 1000 in area 1, was extremely intensively studied with a sample of 20 on each day, except the last, 1st September, when a large part of the available population must have been taken in the sample of 16 recorded.

M. Jurtina, 1946. Area 5. ♀.



M. Jurtina, 1946. Area 5. ♂.



Here, on internal evidence, the death rate was higher in both sexes than the 11 per cent. daily elimination taken as standard. The sex ratio was also appreciably lower, nearly three times as many females as males being taken in all.

At the death rate assumed, the expected total of days exposure to risk of marks recaptured is 278·5 for females against 207 observed, and 202·0 for males against 181. The death rate assumed appears

to overestimate the chances of survival of both males and females, and this perhaps is responsible for the anomalous feature in the table

TABLE 5

Numbers and sex ratio at different dates, area 5

	Numbers captured			Numbers flying, estimated, with 89 per cent. survival		
	Females	Males	Males per cent.	Females	Males	Males per cent.
August 17-20 . .	42	37	46·8	100·0	47·1	32·0
August 21-25 . .	76	24	24·0	154·2	62·5	28·8
August 27-28 . .	26	2	7·1	197·1	73·9	27·3
September 1 . .	12	4	25·0	143·2	33·9	19·1

above, in which the males are given an apparent increase in absolute numbers up to the end of August, whereas it is more probable that, as in area 1, they were really decreasing.

If the estimates are recalculated with 25 per cent. daily elimination, or an expectation of life of only three days, we find

TABLE 6

Numbers flying estimated with 75 per cent. survival, area 5

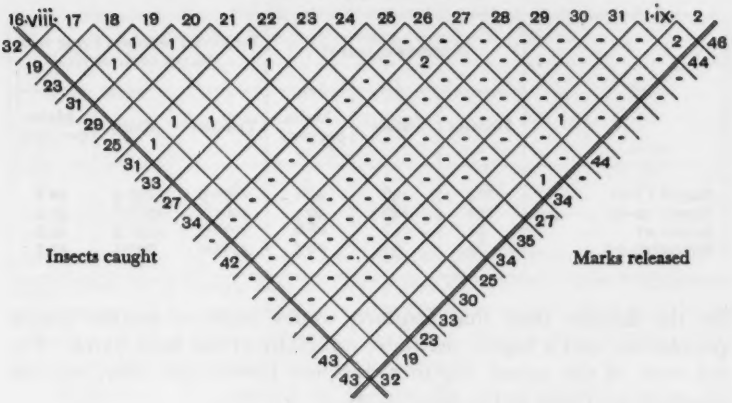
	Females	Males	Males per cent.
August 17-20 . .	67·1	23·4	25·9
August 21-25 . .	75·6	13·7	15·4
August 27-28 . .	53·2	20·6	28·0
September 1 . .	21·6	3·3	13·1

For males the days exposure of marks recaptured now agrees well with the observed 183·2 against 181, though it is still a trifle too high ; for females it is still materially high, 228·0 against 207.

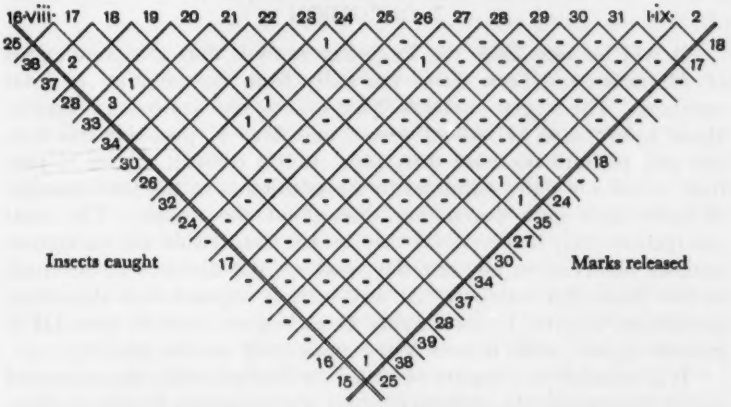
That it would not be reasonable to assign a still shorter life, at least to the males in this area, is shown by the calculated number flying on 1st September having fallen to 3·3 whereas actually 4 were taken. Only 12 females, however, were taken and perhaps there were not so many as 22 available for capture. The suggestion that the female death rate was higher than the male seems paradoxical for this area, where only 30 per cent. of the captures were of males. The expected proportion is, however, only 26·3 per cent. at 11 per cent. daily elimination, and only 21·9 per cent. at 25 per cent. daily loss. Consequently, a somewhat more rapid elimination of females would somewhat improve the agreement with the sex ratios of insects captured.

The population of area 3 was so great that even the allowance of 60 insects to be caught daily led to very few recaptures of marked specimens. Consequently, the internal evidence of survival rate is

M. Jurtina, 1946. Area 3. ♀.



M. Jurtina, 1946. Area 3. ♂.



here weak, and no stress can be laid on the striking deficiency in the apparent longevity of the females. Working at 11 per cent. daily elimination we obtain the results shown in table 7.

For males the total days exposure of the marks recaptured was 76 against 72·64 expected, the death rate assumed thus seems nearly right for males, if anything too high. The total number may therefore have exceeded a maximum of 2500. For females there is a disconcerting discrepancy in that only forty-two days exposure was observed against 65·0 expected. The marks recaptured were, however,

only 14 in all, so that it may well be by chance that most of them had been released only a few days previously. A much higher death rate

TABLE 7

Numbers and sex ratio at different dates in area 3

	Numbers captured			Estimated numbers flying with 89 per cent. survival		
	Females	Males	Males per cent.	Females	Males	Males per cent.
August 17-20 . .	102	136	57·1	1870·9	1259·4	40·2
August 21-25 . .	150	146	50·7	4661·2	2561·8	35·5
August 27 . .	42	17	28·8	3030·3	2531·3	45·5
September 1-2 . .	86	31	26·5	2525·0	733·1	22·5

for the females than that assumed would imply a smaller female population, and a higher sex ratio, especially at the later dates. The sex ratio of the actual captures does not favour this view, nor the observed sex ratios in the other areas.

7. DISCUSSION

It will be seen from the foregoing analysis that the populations of *M. jurtina* on Tean differ markedly from one another in total numbers. The largest numbers flying on any one day being : area 1, about 1400 ; area III, about 7000 ; and Area V, probably less than 250 and perhaps no more than 100. It is a difficult matter to pass from a series of daily estimates to the evaluation of the total number of individuals in a population throughout the season. The total emergences can, however, scarcely be less than twice the maximum number observed on any one day, and are not likely to be so much as five times this number. At any rate, it appears that the whole population in area I considerably exceeds 3000, that in area III it exceeds 15,000, while in area V it cannot easily exceed 500.

It is valuable to compare observed survival-rate with that expected on the assumption of a uniform amount of elimination, for this provides a standard of comparison between different populations. The daily elimination of 11 per cent. may best be used for this purpose. The result is given in table 8 in which, for each population, observation is divided by expectation and expressed as a percentage.

It will be convenient first to consider the two areas which were sampled sufficiently thoroughly to provide trustworthy results : these are I and V. In both sexes the daily elimination rate is markedly higher in the smaller area V than in the larger area I.

Area I is approximately 170 by 130 yards and harboured about 1400 individuals at the maximum, while area V is approximately

150 by 65 yards with a maximum population of less than 250. Thus it will be noticed that the population is relatively denser in area I. This is probably connected with the fact that the opportunities for

TABLE 8

Percentages observed compared with expected survival at 11 per cent. daily elimination [being, (observation÷expectation) per cent.]

Area	Males	Females	Totals
I	107·3	108·2	107·8
III	104·7	64·6	85·8
V	89·6	74·3	80·7

sheltered flight in bad weather will on the average be better in a large than in a small, and probably less diversified, area. This consideration will not only tend to reduce the relative size of the population which a small area can support but also to shorten the average expectation of life of the individuals composing it.

Moreover, the chances of a butterfly straying and being lost, or of being blown away, are considerable when it is close to the edge of a colony but much smaller when well within it. All parts of area V, which is small and narrow, are probably dangerous from this point of view, while a fraction only of the larger and squarer area I will be so. This effect also will reduce the average length of life of *M. jurtina* in area V compared with area I.

The survival of the two sexes is almost equal in area I, but that of the males appears to be somewhat superior in area V. The significance of this is not very clear, but the females are the larger and fly less powerfully. It is possible therefore that they may more frequently be blown away from the relatively exposed area V.

As indicated in the last section, unlike areas I and V, the population in area III was very imperfectly sampled. Owing to its great size, the number of recaptures has proved too small to provide the necessary data even though we marked 60 specimens per day. The close agreement between the survival-rate of males in this area and in area I, where the population also much exceeds 1000, certainly suggests that the elimination is approximately equal in these larger areas. However, as explained on p. 79, no significance can be attached to the very low survival rate of the females in area III since only 14 marks (and specimens, for none of these insects was caught three times) were recaptured. Consequently a chance excess of butterflies recently marked could easily occur and would give the appearance of a high rate of elimination. It will be noticed that none of the results for areas I and V is based upon less than 37 marks recaptured, so that the average time interval between marking and recapturing is tolerably well established.

There is moreover direct evidence for believing that the elimination-rate of females in area III is not unduly high. It must be emphasised that, as shown in section 6, the comparisons between the calculated sex-ratios and those actually observed provide a valuable independent check on our estimates. It will have been noticed that the observed and calculated sex ratios are, in general, in good agreement, while the observed sex-ratio in area III does not suggest a much greater elimination of females than of males; were the female elimination relatively great, the sex-ratio would be higher, especially at the later dates.

Our failure to obtain an adequate sample of the very large population in area III is of importance for the general theory of population sampling. The larger a population may be, the more difficult it becomes to mark enough specimens to obtain a sufficiency of recaptures. The recovery of but few marked individuals may indeed provide a fair general indication of the size of a population, as it has done in this instance, but not of its more detailed properties such as the elimination-rate to which it is exposed. At the other extreme, a very small population cannot easily be sampled owing to the difficulty of capturing enough specimens, marked or unmarked, for the purpose. There is thus an optimum range of population-size for such studies. When engaged in work of this kind, the investigator will have to modify the size of his samples in the light of his results as he proceeds, so as to ensure an adequate recovery of marks. This might prove impracticable in a very large population.

In our work on the moth *Panaxia dominula* (2) we produced evidence to show that chance fluctuations in gene-ratio do not control the evolution of isolated populations of about 1000 or more. On the other hand, though the effects of random survival may be of some importance in populations smaller than this, we suggested that small isolated populations will usually become extinct in periods of time which must be extremely short from an evolutionary point of view.

The present study of *Maniola jurtina* is relevant to this conclusion. We are here able to make sufficiently accurate comparisons of two populations whose approximate size is known; one of above 3000 individuals and one which cannot easily exceed 500. The survival-rate proves to be substantially less in the smaller of them, and this is true for both sexes when treated as separate populations within each colony. Consequently it seems likely that this butterfly would not maintain itself in area V were it not recruited by some leakage of specimens from area III across the barrier presented by area IV.

Small, compared with large, isolated populations are subject to disadvantages of several kinds. Some of these are due to the fact that numerically small colonies tend to occupy areas of less extent than large ones, while several other handicaps from which they suffer

are not dependent upon the size of the inhabited territory. As already suggested, loss from the relatively great perimeter of a small area probably constitutes a serious menace to such forms as Lepidoptera in an exposed situation, as in area V on Tean. Yet it may be of negligible significance in ecological conditions of a different kind; for instance, those experienced by mice in a wood. On the other hand, the less diversified nature of small than of large habitats is a danger of more general application. Among the defects inherent in small populations irrespective of the size of the territory they colonise, two may be mentioned here: the dangers which sometimes result from inbreeding and those which arise from fluctuation in numbers. This latter consideration is of great importance. Such fluctuations are very usual in animal communities, and though they may be safely tolerated by a large population they may reduce a small one to such a low level that recovery becomes difficult or impossible.

There are thus several tendencies which all contribute to make quite small isolated populations, those of materially less than 1000, impermanent units. Yet it is in them alone, not in those of larger size, that chance fluctuations in gene-ratio could possibly contribute to evolutionary change, even if the great difficulties to believing that they would in fact do so, could be set aside.

8. SUMMARY

1. We have studied three populations of the butterfly *Maniola jurtina* on the Island of Tean, Isles of Scilly.

2. These populations are isolated by minor ecological barriers. The amount of interchange between them was determined by marking methods and found to be small.

3. The migration of this butterfly from one island to another appears to be rare, a conclusion confirmed also by marking methods.

4. A quantitative character, spot-number on the hind wings, was studied to determine whether the three populations had diverged measurably in isolation. No indication of this was found. The distribution of these spots proved to be exceptional, being unimodal in the male but bimodal in the female (the heterozygous sex).

5. Judged on spot-number, the female is the more variable sex. Greater female variability has also been detected in the Lepidoptera on a previous occasion: in a survey of 35 species of night-flying moths conducted by two of the authors twenty years ago.

6. The three colonies of *M. jurtina* occupied areas which are severally large (area III), intermediate in size (area I), and small (area V): see map.

7. The number of individuals in these populations was estimated by the method of marking, release, and recapture.

8. The total emergence in area III exceeded 15,000, in area I it exceeded 3000, while in area V it could not much exceed 500.

9. A life-table, giving daily elimination-rates, was calculated for each sex in each population.

10. The data obtained in areas I and V supplied a satisfactory basis for this work, but the recaptures in the largest population were too few to provide corresponding evidence for area III.

11. The survival-rate proved to be substantially less for the population of about 500 (in area V) than for that exceeding 3000 (in area I). This applied to both sexes when treated as separate populations within each colony.

12. An independent check upon these estimates is provided by the comparison between the calculated sex-ratios and those actually observed at different dates.

13. The probability that populations much below 1000 frequently become extinct is considered both theoretically and in the light of these data, and its bearing upon evolutionary theory is pointed out.

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GENETICAL STUDIES IN PEARS

V. VEGETATIVE AND FRUIT CHARACTERS

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Received 3.iii.48

GENETIC studies on cultivated pears have been in progress at Merton since 1931. What concerned sterility and incompatibility has been previously reported upon (Crane and Lewis, 1942; Lewis and Modlibowska, 1942). The present account deals with the inheritance of characters of the leaves, shoots, and fruit. With seedling pears the length of time from seed to maturity varies greatly even within the same family, and many seedlings have not yet borne fruits; hence the data on fruit characters are less complete than those on the vegetative characters. Fifteen diploid varieties have been used as parents. These varieties and their known genetic constitutions are given in table 1.

TABLE 1

Genetic constitutions of varieties

Fertility	RR	SS (Ss) *	hh	ee	GG
Beurré Giffard	RR	ss	Hh	Ee	Gg
Beurré Hardy	Rr	Ss	Hh	ee	GG
Doyenné du Comice	Rr	SS	hh	ee	Gg
Dr Jules Guyot	Rr	Ss	hh	ee	GG
Durondeau	Rr	Ss	hh	Ee	G—
Beurré Clairgeau	Rr	Ss	hh	ee	Gg
Hessle	Rr	ss	hh	E—	G—
Charles Ernest	Rr	ss	hh	ee	GG
Williams' Bon Chrétien	rr	Ss	hh	ee	GG
Laxton's Superb	rr	Ss	hh	ee	G—
Marguérite Marillat	rr	Ss	hh	ee	G—
Glou Morceau	rr	S—	hh	ee	G—
Emile d'Heyst	rr	S—	hh	ee	G—
Conference	rr	Ss (ss) *	hh	ee	Gg

R = summer shoots red — r = summer shoots green or pale yellowish-green
S = leaf margin serrate or crenate — s = leaf margin entire
H = summer shoots hairy — h = summer shoots glabrous or sparsely hairy
E = main leaf midrib eglandular — e = main leaf midrib glandular
G = leaves green or dark green — g = leaves pale green
* Somatic mutations

1. VEGETATIVE CHARACTER DIFFERENCES

Shoot—colour: R—r.—The young, current season's shoots of pears fall into two main colour groups: red and green. In the red group the intensity of the pigmentation varies from a mere tinge to a dark

red or purple colour almost completely covering the young shoot. In all individuals with coloured growth, the colour is influenced by environment. Thus shoots in the central part of a tree are not, as a rule, so deeply coloured as those more exposed. Differences also occur in the colour of the petioles and midribs of the leaves. The colour of the shoot is most clearly seen during the early weeks of growth. Later, colour differences are less distinct owing to the formation of bark, though it is often possible to distinguish green from red coloured individuals even on one-year-old wood, *i.e.* the previous season's growth.

Shoot—hairiness : H—h.—The young shoots of pears differ in their degrees of hairiness : (1) hairy, (2) very few hairs or glabrous. The surface of the young shoots, and also that of the young unfolding leaves, in the hairy class (1) are thickly covered with greyish hairs, and varieties in this class are readily distinguished from those with few hairs in class (2). Differences in hairiness are more readily observed in the early stages of growth. Later as the shoots become suberised, the hairy character becomes obscure.

Leaf—margin : S—s.—The leaves of pears can again be divided into two primary classes, those with a toothed and those with an entire, non-toothed, margin. On the lateral and terminal shoots of the current season's growth the margin of the leaf is often variable, but on the spur leaves the character of the leaf-margin is constant. Within the toothed class the margin varies from sharply serrate to almost crenate, but there is a continuous gradation and, so far, it has not been found possible to subdivide them with confidence.

Leaf—colour : G—g.—The leaves are dark or pale green. Variation occurs within the dark green class, some being darker green than others, but the pale green are always distinct.

Leaf—glands : E—e.—The midrib of the leaf is either free from glands or it is covered with small glands throughout its length. In the early stages of growth they are red, later they turn black. For convenience we have called these two types eglandular and glandular but the eglandular class have a few glands at the base of the leaf. The difference is constant even under diverse conditions and can be readily observed throughout the growing season.

Amongst the glandular varieties used as parents there is great variation in the number of glands along the midrib. Thus Dr Jules Guyot has an average of 179·3 ; Williams' Bon Chrétien 112·8 ; Conference 52·5 ; and Doyenné du Comice 29·4 per leaf.

As shown in table 2 the F_1 progeny has a mean gland number which is always lower than the arithmetic mean of the parents. Most F_1 individuals have fewer glands than either parent. Only in one F_1 family do seedlings occur with more glands than either of the parents ; this is in the family raised from Conference (52·5) × Fertility (89·0). These varieties are rather similar, or at least they

are not so divergent in gland number as are the parents of the other F_1 families.

TABLE 2

Inheritance of gland numbers

Parents	Parental gland numbers	Mean of parents		Mean of F_1
		Arithmetic	Geometric	
Guyot × Giffard .	179·3 × 6·0	92·6	32·8	25·6
Guyot × Conference	179·3 × 52·5	115·9	99·4	89·7
Fertility × Giffard .	89·0 × 6·0	47·5	23·1	23·2
Conference × Fertility .	52·5 × 89·0	70·7	68·3	67·9

Most varieties as table 1 shows are heterozygous in one or more respects. The variety Fertility is the only one homozygous for all five genes, being dominant for three and recessive for two. No varieties have been found to be homozygous hairy HH, or homozygous eglandular EE. Nor has any recessive gg variety been found ; it is probable that they do not occur among cultivated varieties owing to a general lack of vigour being associated with the recessive pale green leaf character.

All the characters so far described have discontinuity with more or less continuous variation on one side, in four cases on the dominant and in one on the recessive side. Thus although they have given mendelian results most of the characters have a polygenic background.

The results obtained from the breeding experiments are given in table 3.

2. INHERITANCE

Mutation.—Two evidences not included in table 2 were found of differences arising within varieties by mutation :—

(i) Beurré Giffard ss × Fertility SS gave abnormal results in respect of the leaf-margin character. Forty-one Ss and 15 ss were obtained when all Ss seedlings were expected. Similarly in a small reciprocal family of 10 individuals, 9 Ss and 1 ss seedlings were obtained. It seems that Fertility must be a mosaic : SS/Ss.

(ii) Several trees of the variety Conference have been used as parents. It was noticed that they differed in their leaf-margins. The majority had an entire, but two had a slightly serrate, margin. Families raised from crossing these two slightly serrate trees with recessive ss varieties, gave approximately equality of serrate and entire. Thus these slightly serrate trees of Conference are heterozygous Ss for the leaf-margin character. Presumably these had given the entire ss by mutation.

3. FRUIT CHARACTER DIFFERENCES

In contrast to the discontinuity of the characters of the leaves and shoots, the range of variation of the characters of the fruit is more or less continuous. Therefore, although care has been taken in recording and also in the classification of the seedlings in the families

TABLE 3

Families from pear crosses grouped according to the segregation types

	No. of			χ^2*		
	families	varieties		Segregation	Heterogeneity	p Heterogeneity
Growth colour—			R r			
RR × (RR or Rr or rr)	12	6	570 : 0	0·0	0·0	...
Rr × Rr . .	8	5	95 : 38	0·894	2·216	·9
Rr × rr . . .	15	8	148 : 156	0·210	10·625	·7
rr × rr . . .	5	4	0 : 147	0·0	0·0	...
Growth hairiness—			H h			
Hh × hh . . .	7	6	82 : 86	0·095	22·803	<·01
hh × hh . . .	45	10	0 : 1011	0·0	0·0	...
Leaf-margin—			S s†			
SS × (SS or Ss or ss)	16	8	537 : 0	0·0	0·0	...
Ss × Ss . . .	12	8	283 : 77	2·059	41·519	<·01
Ss × ss . . .	9	7	127 : 72	15·201	22·541	<·01
Leaf-colour—			G g			
Gg × Gg . .	4	4	77 : 22	0·405	0·449	·9
Leaf-glands—			E e			
Ee × Ee . . .	2	3	123 : 49	1·116	1·697	·2
Ee × ee . . .	8	8	82 : 124‡	8·553	17·268	·02
ee × ee . . .	37	11	0 : 899	0·0	0·0	...

* χ^2 for 1 degree of freedom when p = ·05 = 3·841
† Deficiency of recessives
‡ Excess of recessives

raised, it is not possible, at the present stage of the investigations, to attempt a detailed genetic analysis of fruit characters. Nevertheless differences in segregation can often be correlated with the characters of the parents, and although there is no complete dominance of any fruit character, incomplete dominance is commonly evident.

Size.—The size of the fruit of pears fluctuates as a result of differences in environment, nutrition and other causes. It is impossible to define such characters accurately, and our measurements and determinations must be read as average figures under our conditions. The parent varieties and seedlings, however, have been grown under similar conditions, consequently differences due to these causes are not likely to be considerable. Most of the seedlings dealt with in

this paper have carried fruits for four or more seasons and their shape, size, time of maturity, etc., have been recorded and compared from year to year.

TABLE 4

Inheritance of fruit shape

Parents	Mean	Progeny		
		No. of seedlings	Mean	Variance
Fertility (150) × Giffard (125)	137	53	126·9	8·7
Fertility (150) × Comice (126)	138	42	112·8	8·4
Fertility (150) × Guyot (138)	144	75	121·4	4·8
Fertility (150) × Conference (180)	165	125	142·8	3·7
Conference (180) × Giffard (125)	152	31	143·2	9·6
Conference (180) × Comice (126)	153	45	140·2	15·0
Williams (129) × Conference (180)	154	87	152·0	8·4
Guyot (138) × Conference (180)	159	38	158·5	18·2
Durondeau (131) × Guyot (138)	134	33	133·0	13·2

	S $(x-\bar{x})^2$	D.F.	Mean square	V.R.	p
Within families	267,557·2	520	514·5	22·2	Very small
Between families	91,608·1	8	11,451·0	...	...
Total	359,165·3	528	...	...	...

TABLE 5

Inheritance of fruit size

Parents	Mean	Progeny		
		No. of seedlings	Mean	Variance
Fertility (49) × Giffard (72)	60	53	57·0	24·0
Fertility (49) × Comice (147)	98	42	64·4	16·0
Fertility (49) × Guyot (198)	123	75	83·9	3·5
Fertility (49) × Conference (168)	108	125	63·4	4·1
Conference (168) × Giffard (72)	120	31	57·7	13·9
Conference (168) × Comice (147)	157	45	74·9	30·3
Williams (228) × Conference (168)	198	87	73·7	7·9
Guyot (198) × Conference (168)	183	38	89·1	29·5
Durondeau (188) × Guyot (198)	193	33	75·9	13·6

	S $(x-\bar{x})^2$	D.F.	Mean square	V.R.	p
Within families	462,740·70	520	889·886	7·425	Very small
Between families	52,862·87	8	6,607·859	...	...
Total	515,603·57	528	...	...	...

TABLE 6

Inheritance of fruit length

Parents	Mean	Progeny		
		No. of seedlings	Mean	Variance
Fertility (7·5) × Giffard (7·5)	7·5	53	6·9	·056
Fertility (7·5) × Comice (9·7)	8·6	42	6·8	·049
Fertility (7·5) × Guyot (11·5)	9·5	75	7·7	·028
Fertility (7·5) × Conference (13·0)	10·2	125	7·9	·013
Conference (13·0) × Giffard (7·5)	10·2	31	7·7	·043
Conference (13 0) × Comice (9·7)	11·3	45	8·1	·063
Williams (11·5) × Conference (13·0)	12·2	87	8·7	·030
Guyot (11·5) × Conference (13·0)	12·2	38	9·5	·087
Durondeau (11·5) × Guyot (11·5)	11·5	33	8·0	·043

	$S(x-\bar{x})^2$	D.F.	Mean square	V.R.	p
Within families	1,151·079	520	2·213	...	Very small
Between families	265·629	8	33·204	14·99	...
Total	1,416·708	528	...	...	...

The whole of the data with respect to size and also shape of fruit is not given in this paper, but the hereditary behaviour shown in text figures 1-3 and tables 4-6 is typical of the other families raised.

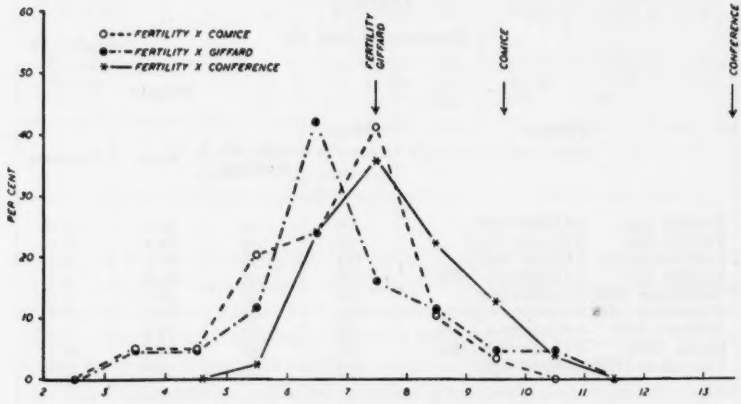

Fig. 1.—Range of variation of fruit length in the progenies from crossing pear varieties.

The mean size of the parents and of the progeny of nine families is given in table 4. The figures denoting fruit size were derived from the formula, *length* × (*radius*)², this although not allowing for differences

in shape gives an approximate measure of relative size. In all families we have raised, the mean size of the fruit of the progeny is smaller than the mean size of the parents.

Shape.—The shape of the fruit also fluctuates subject to the environment, although less so than size. In some cases shape is

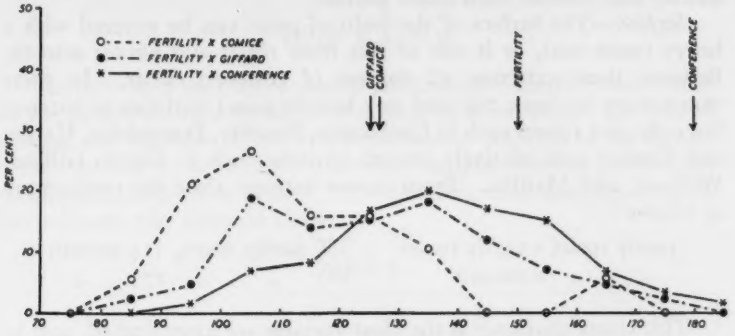

FIG. 2.—Range of variation of fruit shape in the progenies from crossing pear varieties.

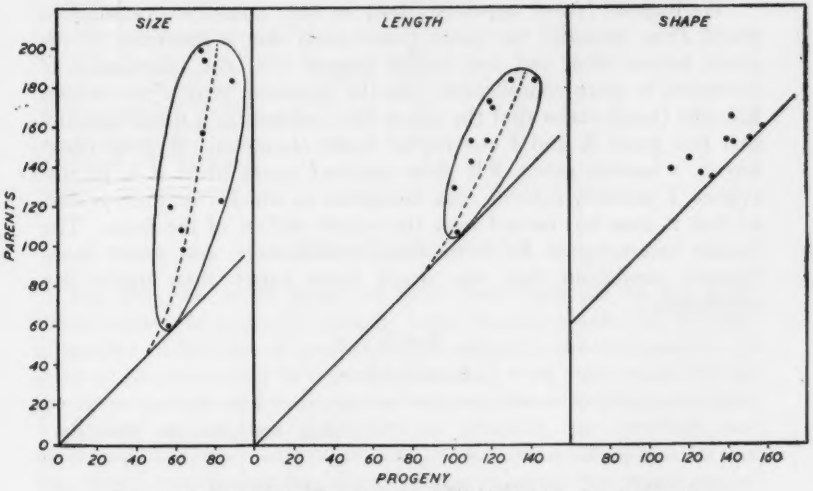

FIG. 3.—Graphs showing the relationship of fruit size, length and shape in parents and progeny from data in tables 4, 5 and 6.

correlated with the number of seeds formed within the fruit. Fruits with few or no seeds are generally more elongated than those with many. The mean shape and mean length of the parents and of the progeny of nine families are given in tables 5 and 6, and the range of variation found in the progeny of three families in the length and shape of the fruits are given in figs. 1-3. The figures for fruit shape

represent the ratio *length/breadth* expressed as a percentage. At 100, the fruits are round, or at least their greatest breadth and length is the same. Above 100, fruits are long and, below 100, flat. The mean length of the progeny is in all cases lower than the mean of the parents, and hence as shown in fig. 2, a majority of the progeny are shorter and rounder than either parent.

Surface.—The surface of the fruits of pears can be covered with a heavy russet coat, or it can be free from russet and almost smooth. Between these extremes all degrees of russeting occur. In these experiments we have not used any heavily russet varieties as parents, but only part russets such as Conference, Fertility, Durondeau, Hardy, and Comice and relatively smooth varieties such as Guyot, Giffard, Williams, and Marillat. From crosses between these the results were as follows :—

partly russet × partly russet	166 partly russet,	114 smooth
„ „ × smooth	187 „ „	273 „

This russet character is the most variable we have studied, and in particular in the partly russet kinds it appears to fluctuate widely according to environment.

Wellington (1913) reported that, in the European varieties of pears, *Pyrus communis*, the green (non-russet) skin is dominant to the russet brown skin, but our results suggest that the inheritance of russetness is more complicated. In the Japanese pear, *Pyrus serotina* Kikuchi (1930) states that the russet skin behaves as a dominant and that two genes R and I govern the russet character. R gives russet brown, r smooth green, RR gives constant russet when R is heterozygous, I partially inhibits cork formation to which russetness is due, so that it does not extend over the whole surface of the fruit. The double heterozygotes Rr Ii fluctuate considerable, and under moist climatic conditions they are much more russet than under dry conditions.

TABLE 7

Season of ripening

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Fertility (Oct.) × Giffard (Aug.) . .	29	18	14	...	...	...
Fertility (Oct.) × Guyot (Sept.) . .	11	44	10	...	...	...
Fertility (Oct.) × Conference (Oct.-Nov.)	...	40	38	1	...	...
Fertility (Oct.) × Comice (Nov.) . .	1	10	13	1	...	...
Conference (Oct.-Nov.) × Guyot (Sept.) . .	6	14	4	...	...	...
Conference (Oct.-Nov.) × Marillat (Sept.) . .	10	25	15	2	...	1
Conference (Oct.-Nov.) × Williams (Sept.) . .	7	42	18	5	...	1
Conference (Oct.-Nov.) × Durondeau (Oct.-Nov.)	...	14	7	4	...	1
Conference (Oct.-Nov.) × Comice (Nov.) . .	...	22	19	1	...	...
Comice (Nov.) × Williams (Sept.) . .	...	4	10	1	...	...
Comice (Nov.) × Clairgeau (Nov.-Dec.) .	...	8	16	...	...	2
Durondeau (Oct.-Nov.) × Guyot (Sept.) . .	3	21	12	1	...	...

Season of ripening.—The season of ripening of the fruit of the parent varieties and of a number of seedlings is given in table 7. The mean season of ripening of the seedlings is in all families much earlier than that of the parents and the proportion of seedlings which are later than the latest ripening parent is remarkably small.

4. FRUIT FLAVOUR

The flavour of the fruit of pears is particularly difficult to determine. In the early maturing seedlings it is often ephemeral and the time of picking and testing is of the first importance. Analysis of the flavour of the seedlings we have raised is far from complete but our preliminary classification is given in table 8. The best flavoured seedlings occur in the aromatic and sub-acid classes.

TABLE 8

	A Sweet	B Aromatic	C Sub-acid	D Acid
Fertility A × Giffard B	48	2	14	1
Fertility A × Guyot A	52	0	17	1
Fertility A × Conference A	61	12	9	2
Fertility A × Comice B	20	5	6	1
Conference A × Guyot A	22	5	5	2
Conference A × Marillat B	31	9	13	1
Conference A × Williams B	53	15	5	1
Conference A × Durondeau C	12	5	4	3
Conference A × Comice B	25	12	5	2
Comice B × Williams B	7	5	3	2

5. DISEASE RESISTANCE

For the past seven years we have been indebted to the Lord Wandsworth Agricultural College, Long Sutton, Hants, for growing a number of families of seedling pears which it was not possible to grow at Merton owing to lack of land. The trees were raised during the years 1936-39, and were planted at Long Sutton in February 1941.

Owing to war-time difficulties no spraying, nor pruning, nor manuring, nor other cultivation was carried out after 1943. In one way these conditions have added to the value of the experiments: the ravages of disease were unchecked. In particular, pear scab, *Venturia pirina*, which is probably the most troublesome disease of cultivated pears, was rampant throughout the plantation. This showed us the incidence of the disease in the different families and hence the genetic value of the parental varieties in regard to scab resistance. Amongst the seedlings there was great variation in the severity of the attack. In a few families, a small proportion of the seedlings were free from scab, and presumably immune from the disease.

The seedlings were recorded and classified according to the severity of scab attack in classes as follows :—

1. Extremely severe ; 2. severe ; 3. moderate ; 4. slight ; 5. scab free.

TABLE 9

Parents of the families and numbers of seedlings in the classes of scab resistance

Parents	Classes					Total	Scab-free per cent.	Dead
	1	2	3	4	5			
A. Giffard × Conference .	2	6	9	7	6	30	20·0	3 *
B. Giffard × Fertility .	60	55	33	22	8	178	2·4	4
Giffard × Durondeau .	26	48	61	18	0	153		5
C. Conference × E. d'Heyst .	17	29	2	1	0	49	2·5	10 *
Conference × Hardy . .	23	39	25	20	4	111		1 *
D. Hardy × Conference .	42	10	9	0	0	61	1·7	1
Comice × Conference .	1	11	5	6	2	25		3 *
Williams × Conference .	0	2	5	0	0	7		4 *
Hessle × Conference .	8	8	6	0	0	22		0 *
E. Comice × Durondeau .	21	8	0	0	0	29	0·0	0
Durondeau × Comice .	25	6	2	0	0	33		8 *
Fertility × Guyot . .	14	3	0	0	0	17		0 *
Comice × Glou Morceau	9	19	16	0	0	44		0
Comice × Williams .	2	8	2	1	0	13		1 *
Marillat × Durondeau .	0	1	3	1	0	5		12 *
Hardy × Comice .	20	49	13	0	0	82		2
Hardy × Glou Morceau	24	39	13	0	0	76		4
	294	341	204	76	20	935	...	58

* Quince stock : other families, own roots

Fifty-eight trees died, 42 of these were in the families on Quince "C" rootstock, a mortality of 11·8 per cent. ; 16 were in families growing on their own roots, a mortality of only 2·5 per cent. It is, therefore, probable that the high mortality in the families on Quince "C" was mainly due to stock-scion incompatibility.

The proportion of seedlings within the different classes varied, but in 13 out of the 17 families more than 50 per cent. were in the severely attacked first two classes. The 20 scab-free seedlings all occurred in families where either one or both parents were the varieties Conference or Beurré Giffard. But not all the families where one of these varieties was a parent gave resistant seedlings.

Conference, the most widely grown pear in this country, has long been recognised as being to some extent a scab resistant variety. Giffard is less grown and its degree of resistance to scab is not known.

The families in table 6 are arranged in five classes :—A are those between Giffard and Conference. B and C are those where Giffard

or Conference was used as a female parent, D those where Conference was used as a male parent, and E those where neither Giffard and Conference were used.

The differences in proportion of scab-free seedlings between the five classes suggests that Giffard and Conference have a high resistance-giving capacity. At the same time the absence or low proportion of scab-free seedlings when Conference is used as male and particularly in the reciprocal cross with Hardy indicates that scab resistance depends partly on a cytoplasmically transmitted determinant. It remains to be discovered how permanent this determinant is.

Beurré Giffard has narrow, stiff and somewhat hairy leaves. The seedlings raised from Beurré Giffard × Fertility were classified for leaf shape as follows : (1) broad ; (2) moderately broad ; (3) moderately narrow ; (4) narrow.

There were 178 seedlings in this family and the number in these different classes were :—

Classes	(1)	(2)	(3)	(4)
Seedlings scab-infected . .	7	60	103	8
Seedlings scab-free . .	...	*1*	*3*	*4*

The distribution of the 8 scab-free seedlings in the family is shown in italics. The stiff narrow leaf, therefore, appears to be associated with resistance to scab.

6. DISCUSSION

The characters of the leaves and shoots of pears which we have studied have shown discontinuous variation. Dominance occurs and they show the expected segregation ratios for single factor differences. The characters of the fruit on the contrary have shown continuous variation. This probably results from the action of a number of genes whose expression in the phenotype is cumulative. The results with pear scab suggest that genetical resistance to scab is determined by a certain balance of genes having small but cumulative effects.

The hereditary behaviour of resistance to scab in pears is in several respects similar to the inheritance of resistance to the attack of the woolly aphis, *Eriosoma lanigerum* in apples, *cf.* Crane (1937) and Crane *et al.* (1936).

It may be recalled that in our studies in cultivated apples (Crane and Lawrence, 1933 ; Lewis and Crane, 1941) discontinuity, even in vegetative characters, was far less frequent than in pears. Moffett (1934) found that secondary association of chromosomes was frequent in apples but rare in pears. Thomas and Revell (1946) have concluded that secondary association results from heterochromatic attraction of

chromosomes with corresponding segments of heterochromatin; secondary association in this way indicates homology.

It is generally agreed that two or more species have taken part in the origin of the cultivated apple while the origin of the cultivated pear is simpler. These differences between apples and pears doubtless account for the differences both in chromosome behaviour and in mode of variation.

Accounts all agree that the fruits of truly wild pears, *Pyrus communis*, from which our European cultivated varieties are derived are small, nearly round, hard, gritty, sour, astringent and late ripening. Selection has been towards larger and longer fruits with softer and sweeter flesh and an absence or reduction of grit cells. In some of the characters we have analysed, such as size and length of fruit there has been in the progeny a pronounced shift towards the characters of the wild type. In other characters, such as texture, sweetness and season of ripening no shift in this direction occurs. It is probable that selection for these characters of the fruit has been more rigid than selection for mere shape and size. In addition these characters, which together determine fruit quality, appear to be closely correlated. Sour and astringent pears are usually late ripening and in selecting for a reduction of sourness and astringency, selection has at the same time been towards early ripening.

Early ripening in pears appears to be correlated with a fleeting or insipid flavour since most of the early-ripening individuals we have raised, and also a high proportion of the early-ripening varieties in cultivation, are of this kind. Though not quite so pronounced the same situation is found in apples; the desirable combination of a degree of sweetness and acidity with the maintenance of flavour for an appreciable time is rare with early ripening in both apples and pears. It seems that the highest qualities of flavour are bound up with a certain degree of acidity and that this is associated with a slower development and hence later ripening.

Selection for desirable fruit characters in pears has been going on for centuries, but it has been much slower since pears are slow to reach maturity and also commonly vegetatively reproduced, than it has been in crops which are annually reproduced by sexual reproduction.

7. SUMMARY

Inheritance of the characters of the leaves, growth and fruits of pears have been studied, also of resistance to pear scab *Venturia pirina*.

In pears, unlike apples, five simple mendelian differences of the leaves and growth were found. These we have used in a classification of cultivated varieties.

This difference between pears and apples is related to differences in secondary chromosome association and in phyletic origin.

Many quantitative differences, especially in fruit characters, were found to have a polygenic basis.

Resistance to pear scab also has a polygenic basis. In addition there was a reciprocal difference in crosses. The carrier of resistance was less effective on the male side which suggest that the cytoplasm may take part in the inheritance of resistance.

In fruit size and shape seedlings from cultivated varieties preponderate towards wild type.

We are indebted to Mr A. G. Brown and Mr P. Day for assistance in these experiments.

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REPRODUCTIVE VERSATILITY IN *RUBUS*

III. RASPBERRY-BLACKBERRY HYBRIDS

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THE Veitchberry $4x = 28$ and the Mahdiberry, $3x = 21$, were both raised from a cross between the hedgerow Blackberry *Rubus rusticanus*, $2x = 14$, and tetraploid forms of the raspberry *Rubus idaeus*, $4x = 28$. No diploid hybrid, the result of crossing diploid forms of these two species, appears to be known and attempts we have made to cross them have entirely failed. It therefore seems that seeds are formed only when an unreduced germ cell from one or both diploid parents takes part in fertilisation or when a polyploid form of *R. idaeus* is one of the parents. That unreduced germ cells occasionally occur in *R. rusticanus* is evident, not only from the Veitchberry, but also from the origin of the John Innes Berry, $4x = 28$, which was derived from *R. rusticanus*, $2x = 14$, crossed *R. thyrisiger*, $4x = 28$ (Crane and Darlington, 1927).

In 1935 a plant of the Mahdiberry growing at Merton sent up a very vigorous basal shoot which was found to be pentaploid $5x = 35$. This shoot was removed, but although care was taken it was not possible to be certain that it was attached to the original Mahdi. It is thus derived either as somatic mutation or by segregation in a seedling. Plants derived vegetatively from this shoot are much more vigorous, produce larger fruits and when provision is made for cross-pollination they fruit more freely (fig. 1). The undersides of the leaves of the pentaploid are not so densely hairy as those of the triploid, but apart from this and the increase in size, which is proportionate in all organs, they seem indistinguishable.

Most cultivated varieties of raspberry are heterozygous. Thus the variety Lloyd George with red fruits, red prickles, and hairy growth, gives seedlings with yellow fruits, green prickles, and glabrous growth when selfed. Similarly, the red-fruited variety Superlative with hermaphrodite flowers, in addition to giving yellow fruits, also gives male and female forms by segregation of recessive genes (Crane and Lawrence, 1931). Other genes which suppress pollen development also occur in raspberries (Lewis, 1939).

The Veitchberry combines the characters of its parent species. For example its fully developed leaves are partly pinnate as in *R. idaeus* and partly palmate as in *R. rusticanus*. Upon selfing, its offspring vary only in minor respects such as the colour of the petals and prickles; there is no approach to either of the parental forms. Upon crossing with *R. idaeus* and other species, the behaviour of the Veitchberry is again typically that of a species, but exceptionally a few aberrant forms have appeared in the progeny. All this suggests the general suppression of segregation, as in *Primula kewensis*, through the pairing of like chromosomes.

There are two forms of the Veitchberry; they differ in pollen development, one produces pollen abundantly, the other very sparsely and gives a proportion of the first form on selfing. Whether this difference arose somatically or sexually is not clear, but it is no doubt due to the action of the pollen-inhibiting genes found in the raspberry parent.

From crossing the Veitchberry as female with two diploid forms of *R. idaeus*: (1) the variety Lloyd George and (2) a male seedling from the variety Superlative, we raised two families, one of 118, the other of 126 seedlings. In both families variation occurred in petal and prickle colour and in other minor respects. But, apart from this variation, the result of the heterozygosity of the raspberry parent, 237 out of the 244 seedlings (97·2 per cent.) were intermediate between the parents, and of a uniform type in habit of growth and other major characters.

They were all highly sterile.

Several of them were cytologically examined and found to be triploid, $3x = 21$.

The others, four plants in one family and three in the other were strikingly different. They were all of reduced size. Three of these are shown in plate . BB was very similar to its original blackberry grandparent *R. rusticanus*. RR was typically *R. idaeus*. RB was intermediate between these two species; it was like the Veitchberry but with slenderer and less robust growth, see fig. 2. The other four plants were three blackberry and one intermediate. The raspberry-like seedling was fertile, the others infertile, some of the blackberries entirely so.

The three illustrated seedlings were found to be diploid $2x = 14$ and the other four must have been the same.

To elucidate the origin of these exceptional plants and assess the chromosome relationship between blackberry and raspberry, one of us (P.T.T.) examined the plant RB, the Veitchberry, and other allied *Rubi* shown below.

Veitchberry × Lloyd George $2x = 14$ . .	RB
Veitchberry × Lloyd George $3x = 21$ . .	RRB
Auto-triploid Raspberry $3x = 21$ . . .	RRR
Mahdiberry $3x = 21$	RRB

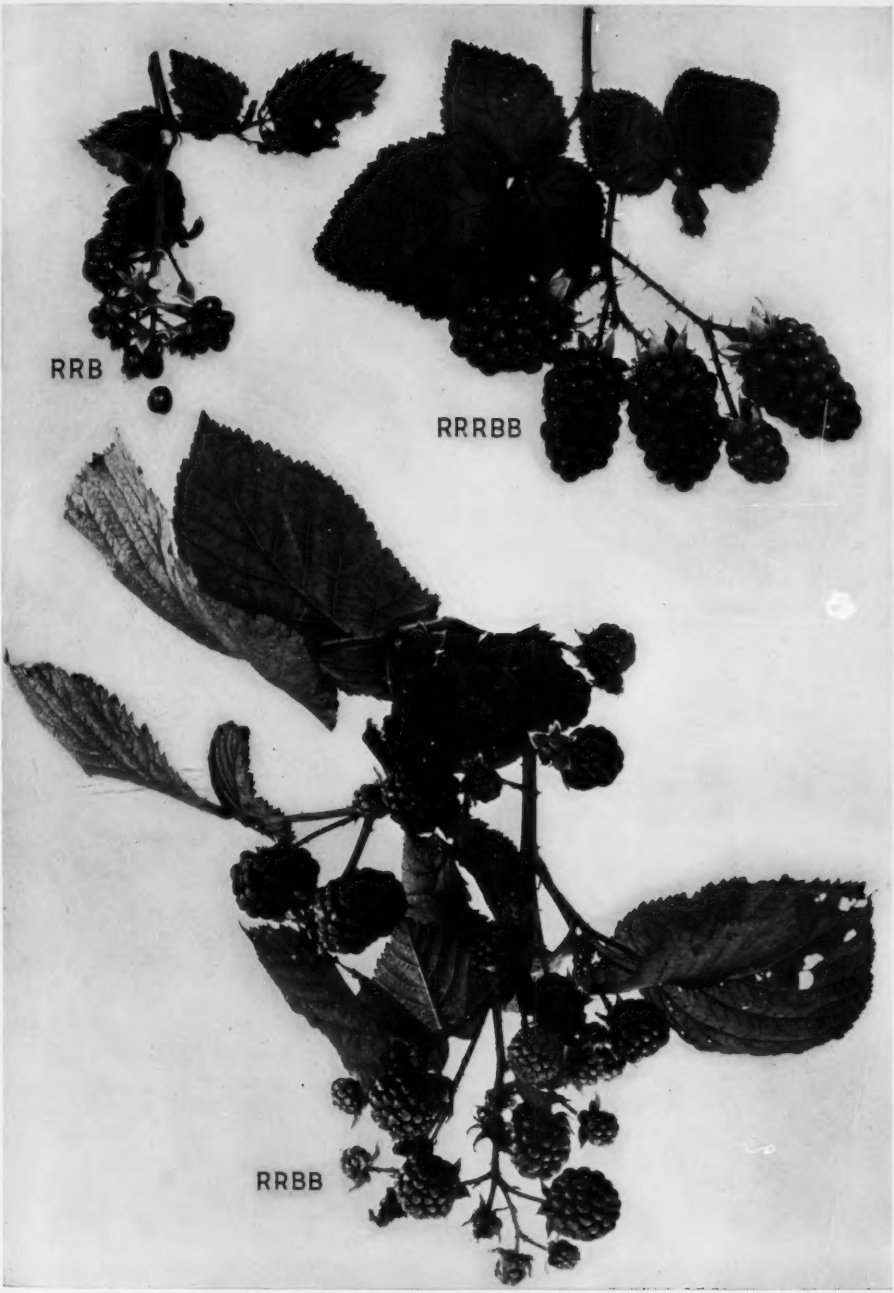


FIG. 1.—The triploid Mahdi RRB, pentaploid Mahdi RRRBB and the tetraploid Veitchberry RRBB. Reduced to $\frac{2}{3}$.

Auto-tetraploid Hailshamberry $4x = 28$. RRRR
Veitchberry $4x = 28$, pollen sparse . . RRBB
Veitchberry selfed $4x = 28$, pollen abundant . RRBB

The Veitchberry used in these studies was the sparse pollen form. The anthers usually fail to develop and those that do develop more or less normally contain only a small proportion of germ tissue—isolated islands of pollen mother-cells are found amongst the somatic tissue. Evidently some physiological disturbance in the young differentiating anther causes some of the potential mother-cells to remain as somatic cells. The degree and type of chromosome pairing did not materially differ from that of the form with normal anther and pollen development which was also examined.

Chromosome association at meiosis in these two plants and in the auto-tetraploid raspberry Hailshamberry, are compared in table 1.

TABLE 1

	Veitchberry $4x = 28$						Hailshamberry $4x = 28$				
	Types of configurations				No. of nuclei		Types of configurations				No. of nuclei
	IV	III	II	I			IV	III	II	I	
	1	0	12	0	5		6	1	0	1	1
	0	1	12	1	4		5	1	2	1	1
	0	0	14	0	2		4	1	4	1	1
	1	0	11	2	7		4	0	6	0	1
	0	0	12	4	4		3	0	8	0	1
	0	0	11	6	6		3	0	6	4	1
	0	0	10	8	2		2	1	8	1	2
	0	0	9	10	1		2	0	9	2	1
	...	...	...	...	...		1	1	8	5	1
Total .	12	4	356	103	31	Total .	31	6	59	16	10
Average	0·4	0·1	11·5	3·1	...	Average	3·1	0·6	5·9	1·6	...

It seems that the Veitchberry differs cytologically from the autotetraploid raspberry in three respects: (1) the number of quadrivalents is lower, 0·4 per nucleus in the Veitchberry, compared with 3·1 per nucleus in the raspberry, (2) the number of univalents is higher and (3) the range in number of univalents is also much higher in the Veitchberry than in the tetraploid raspberry.

Little is known of the way in which raspberry and blackberry chromosomes have become differentiated from one another, because no direct diploid hybrid between the two species has been produced and available for study. The information we have, has been obtained somewhat indirectly from the study of polyploid hybrids. For example in the loganberry, *R. loganobaccus*, which we have shown to have

two sets of raspberry and four sets of blackberry chromosomes, there is little if any pairing between the raspberry and blackberry chromosomes (Crane and Thomas, 1940). On the other hand in the Madhiberry the degree of chromosome association is only slightly less than in an autotriploid raspberry (table 2).

TABLE 2

Frequencies of cells with different numbers of bivalents or trivalents at meiosis in diploid and triploid forms of Rubus

Plant	Formula	0	1	2	3	4	5	6	7	Total	Average
Veitchberry 2x .	RB (II)	...	3	2	7	13	16	10	3	54	4·5
Mahdiberry 3x .	RRB (III)	7	5	11	15	10	6	6	6	66	3·3
Raspberry 3x .	RRR (III)	...	...	2	5	11	19	13	10	60	5·0

It, therefore, appears that raspberry and blackberry chromosomes are sufficiently differentiated to compel self-pairing when there are two sets of each in the hybrid but, when there is no opportunity for differential pairing as in the diploid, and to a lesser extent in triploids, the two types of chromosomes can pair with very little restriction.

Chromosome pairing in the Veitchberry is not that expected of a raspberry-blackberry tetraploid hybrid. Quadrivalents should be less frequent than in the auto-tetraploid but the wide range in number of univalents is not characteristic of an allo-tetraploid. We, therefore, suggest that the failure of pairing is genotypically controlled.

The exceptional diploid plants RB which occurred in the family Veitchberry × Lloyd George Raspberry enabled us to test this conclusion (table 2). There chromosome association ranges from complete pairing to almost complete failure of pairing. This kind of behaviour is what has been observed in pure species where the failure of pairing is due to the presence of a single gene. This interpretation would agree with our earlier conclusion that chromosome pairing in the Veitchberry itself is subject to genic interference.

The seven anomalous diploids must all have originated from the embryo sac of the Veitchberry, the male taking no part in fertilisation. In the BB plants it seems that the chromosomes from the Veitchberry were exclusively blackberry, in RR exclusively raspberry and in RB presumably one set of each.

Regular bivalent formation with autosyndesis (self-pairing) would give embryo sacs with the chromosome constitution RB. Haploid parthogenesis with this type of chromosome behaviour would thus lead to the intermediate type of plant. Segregation to give rise to embryo sacs of the constitution RR or BB for all seven chromosomes would demand complete quadrivalent formation with an orientation at the first metaphase of meiosis, such as would occur only once in 128 times. But our analysis of chromosome behaviour shows that

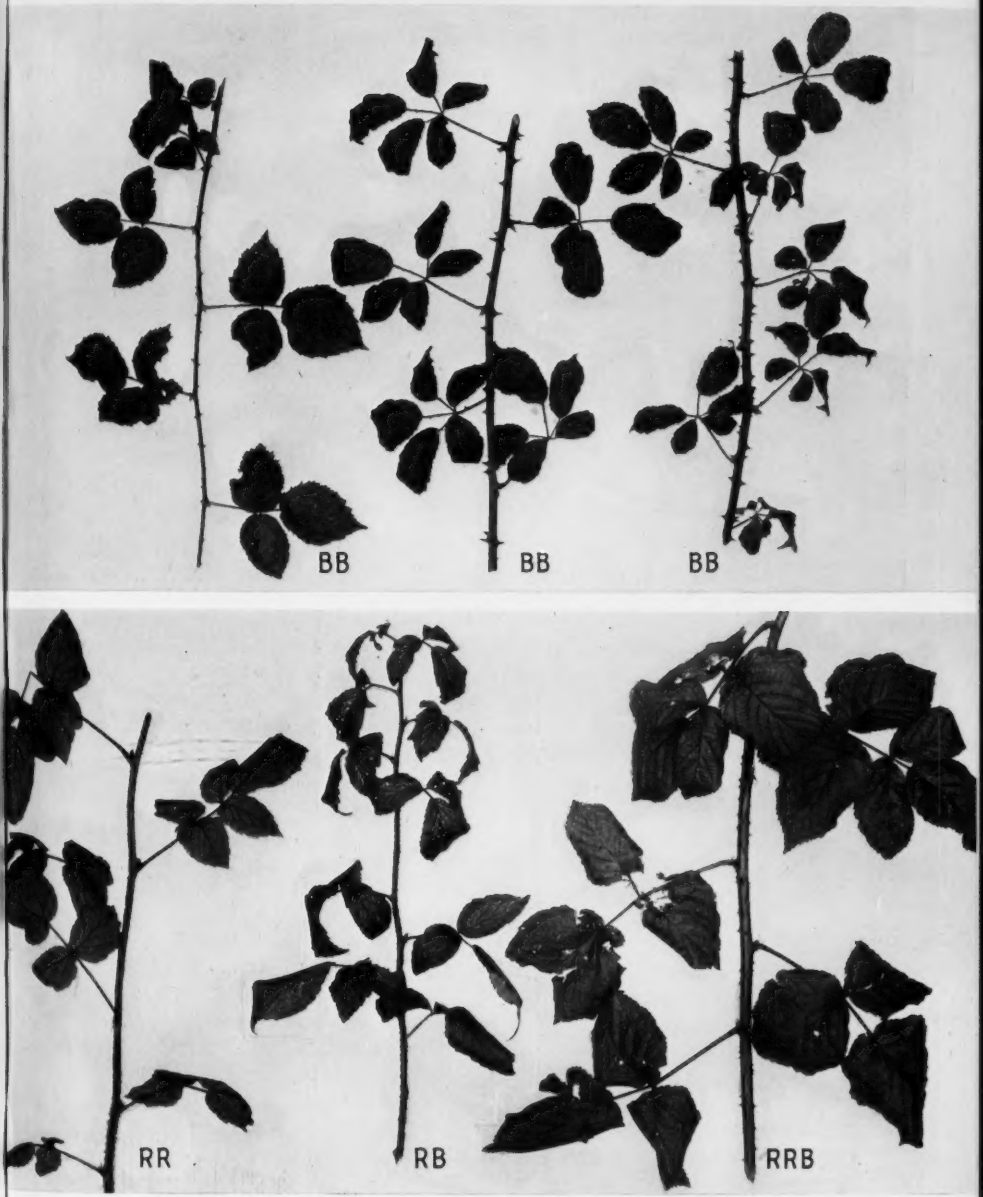


FIG. 2.—Diploid blackberries BB, diploid raspberry RR, diploid intermediate RB and sexual triploid RRB, derived from Veitchberry RRBB crossed raspberry RR. Reduced to $\frac{3}{10}$.

quadrivalent formation is infrequent in the Veitchberry. Alternatively we may visualise the origin of the BB and RR embryo sacs as being derived from a random separation of unpaired chromosomes where there has been a high proportion of univalents.

SUMMARY

1. The Veitchberry $4x = 28$ was derived from *R. rusticanus* $2x = 14$ and a tetraploid form of *R. idaeus* $4x = 28$. Upon selfing there is no approach to either parental forms, it breeds true to its intermediate character.

2. Upon crossing with *R. idaeus* the behaviour of the Veitchberry is again typically that of a species, but exceptional diploid forms have appeared in the progeny : four blackberry-like, two intermediate but slender and one raspberry-like.

3. The chromosomes of the blackberry and the raspberry are differentiated and normally pair among themselves when two sets of each are present in the hybrid.

4. With less opportunity for differential pairing as in the Mahdiberry $3x = 21$ the chromosomes of the two types can pair effectively with one another.

5. The allotetraploid Veitchberry behaves exceptionally in that there is considerable failure of pairing. This failure appears to be due to genotypic control since no structural differences were observed between the raspberry and blackberry chromosomes.

6. One of the intermediate diploid plants from the Veitchberry similarly behaved like an asynaptic diploid.

7. It is concluded that seven diploids in the progeny from Veitchberry $4x = 28$ crossed raspberry $2x = 14$ have originated parthenogenetically from the embryo sacs of the Veitchberry.

APPENDIX

ON AN INTEGRATED SPECIES DIFFERENCE

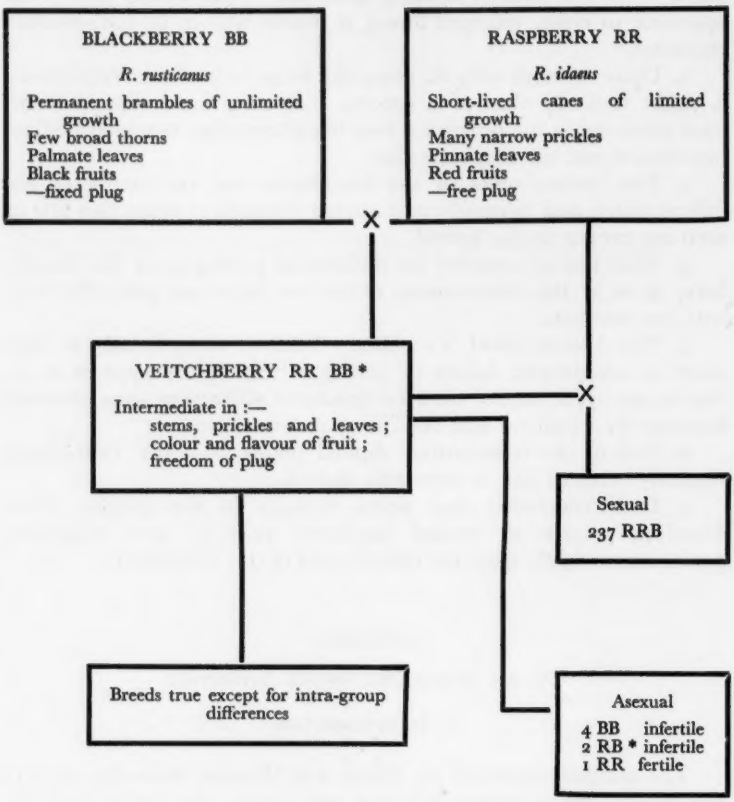
C. D. DARLINGTON

The families examined by Crane and Thomas from the cross of Veitchberry by raspberry fall into two classes, the sexual and the asexual. If these classes are separated and compared two apparently contradictory conclusions follow.

In the first place there is a segregation of species differences in the diploid asexual progeny which is evidently genuine. But the three types of diploid are those expected only if the raspberry-blackberry difference is behaving as a unit in inheritance. Now this difference, as the diagram shows, is an elaborate one. It must obviously depend on numerous mutually adapted gene changes. The recombination of

these in diploids is prevented in the European flora, both wild and cultivated, by the elimination of all diploid hybrids. We now see that it is also prevented in polyploids by some other, even more fundamental, condition. This condition must be the integration of all the differences as a block within which no crossing-over occurs.

Diagram to show the contrasted effects of segregation on the raspberry-blackberry difference in sexual and asexual families of the hybrid



* Genotypic failure of pairing at meiosis in pollen mother cells

In the second place the eggs of the Veitchberry in sexual families (selfed as well as crossed) show no segregation of the differences between its parental species. If such segregation occurs the homozygotes are eliminated. The eggs in asexual families on the contrary show, not merely segregation, but an excess of homozygotes : it is the heterozygotes that are eliminated.

This apparent contradiction is resolved by the cytological evidence. The frequency with which any one chromosome of the seven types enters into a quadrivalent in the Veitchberry is 0·39/7 or 5·5 per cent. of the cells. A quadrivalent will give RR—BB segregation in about half these cells and of them half will suffer loss of laggards and die, leaving 0·7 per cent. of RR gametes and 0·7 per cent. of BB, or one in 140.

Thus one in 140^2, or about 20,000, selfed seedlings of Veitchberry should show segregation of each of the pure types, a possibility which has not been tested.

On the other hand the 0·7 per cent. segregation will be enhanced in the asexual families by the elimination of the diploid heterozygotes. This elimination is independantly shown by the failure of crosses between the diploid species—except in the production of polyploids such as the Veitchberry and the Mahdi. Thus the ***diploid*** elimination conceals the heterozygotes and the ***tetraploid*** recombination conceals the homozygotes.

The physiological integration of the raspberry and blackberry types in the *Rubus* cell must be combined with the basis of genetic isolation between the two groups. This isolation appears to be twofold : it is shown by the non-viability of direct diploid hybrids and also by the sterility of the indirect diploid arising from parthenogenesis of the tetraploid. This sterility strongly expressed appears as morphological male-sterility, and weakly expressed as reduced pairing at meiosis ; in all gradations it is genotypically controlled.

The non-viability of the diploid hybrid is also clarified by the present experiment. It seems not to be inherent since the diploid RB heterozygote can be raised in this indirect way. It probably depends on an error in the embryo-endosperm-ovary relationship in the diploid first cross which is no longer completely effective as a means of eliminating the diploid progeny of the tetraploid hybrid.

One consequence of this unitary difference is in the interpretation of the Mahdi sport. The pentaploid has evidently arisen by doubling (vegetative or sexual) in the triploid followed by the loss of one chromosome set, *i.e.* seven complementary chromosomes taken at random from the 42.

The maintenance of the same external form with such a change of chromosome complement was preposterous in terms of a dispersed difference : it becomes intelligible in terms of an integrated one. To suppose that the 2 to 1 raspberry-blackberry proportion of the original Mahdi might persist in its giant mutant as a 3 to 2 proportion, avoiding any external qualitative variation by a precise regulation for all seven chromosomes of the set was too much. But to suppose that such a change could take place for a single member of the set although astonishing, is not utterly unreasonable. It now therefore becomes necessary to suppose that extensive changes hitherto known only in the " vegetative regulation " of the mosses (Wettstein, 1924)

may take place in mitotic chromosome numbers, subject to mechanical loss of chromosomes and physiological selection of cells.

How is the raspberry-blackberry difference constituted ?

In *Oenothera* we know how interchange makes it possible to hold together in the interchange hybrids large or complex differences lying in the seven different chromosomes. But here there is no evidence of interchange. All the differences must therefore lie in one chromosome.

We have to visualise a single super-gene consisting of many parts with mutually adjusted effects. These parts must be recombinable within each group as has been shown both for the prickles of the blackberry by Crane and Darlington (1927) and of the raspberry by Lewis (1940). It is in this way that an enormous range of species has been produced in each group, limited in number indeed only by the supply of materials and names that will enable us to preserve and describe them. On the other hand in crosses between the two groups the ultimate differences are held together. The diploid blackberry segregates arising from the Veitchberry have not merely the general blackberry character ; they have the very prickles of their *rusticanus* grandparent.

Such a system can arise by the inversion of a chromosome segment. In most natural populations there are inversions floating which occasionally will chance to include (or, shall we say, *collide* with) groups of mutually adapted genes capable of becoming a focus of fruitful discontinuity. And the coincidence having occurred, the resulting super-gene will evolve in the way that has been described from its successive stages by Darlington and Mather (1949).

Thus, in a variety of ways, we have evidence of the basis of the chief genetic divarication within the genus *Rubus*. The fact that such an elaborate divarication is effective only at the diploid level shows that it is at this level that the long-term evolutionary processes have been at work, the polyploids being a very recent, indeed perhaps a post-glacial, novelty.

In the present super-gene difference we can already see two stages of development. The integration of a block by suppression of crossing-over, and the non-viability of the diploid hybrid, both prevent recombination. But the integration of the block must have come first since the non-viability of the hybrid between two groups automatically brings to an end this organised divergence.

The closest analogy to the present situation is in the speltoid and fatuoid complexes of the cereals. The difference is that these complexes have arisen in cultivated plants and in hexaploids. Moreover they distinguish smaller systematic groups and lead to no inviability in the hybrid. The *Rubus* super-gene is therefore presumably a much older complex.

The problem that now arises is thus to compare the character and scope of the discontinuity between different sections of the main raspberry and blackberry groups in different parts of the world.

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PRODUCTION OF LETHAL MUTATIONS IN THE FUNGUS CHÆTOMIUM GLOBOSUM BY MONOCHROMATIC ULTRA-VIOLET IRRADIATION

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INTRODUCTION

It has been shown (McAulay and Ford, 1947) that the nature and distribution of mutations of *Chætomium globosum* produced by monochromatic ultra-violet irradiation are different for different ranges of wave length. For this and other reasons, it appears that more than one primary absorption is involved in mutation production in this fungus, and that there is some evidence that one of these absorptions takes place in a protein. This result is not in harmony with the majority of experiments described in the literature and it appears desirable to confirm it and make a more detailed investigation than is possible with the very slow experiments that are necessary with the mutants described in the above paper.

It has been shown (Ford, 1948) that lethal colonies are produced by ultra-violet irradiation of *Chætomium globosum* and because these can be recognised early, it is possible to obtain more data about them than about the *macroscopic* mutants investigated earlier. In the above paper (Ford, 1948) it is shown that small colonies that do not grow beyond 1 mm. in diameter are produced by ultra-violet and X-irradiation, and also appear through many generations in the progeny of mutations produced by ultra-violet irradiation. For these and other reasons it is claimed that these lethal colonies can be regarded as mutations, and they have been named by Ford *microscopic* mutants.

In this paper, experiments are described and curves drawn which show the relationship between the numbers of microscopic mutants produced and dose at several different ultra-violet wave lengths.

The dose mutant production relation has been studied in more detail at one wave length (2804 A.U.), and additional experiments made to see if there were any variation with intensity of radiation. An estimate has been made of the number of quantum hits necessary to produce a microscopic mutant, and on the assumption that mutation is brought about by quantum absorption in a sensitive spot an estimate has been made of the absorption coefficient at different wave lengths of the radiation in this spot.

EXPERIMENTAL ARRANGEMENT AND ABSORPTION OF THE RADIATION IN THE MATERIAL OF THE SPORE

The arrangement used for irradiation is similar to that described by McAulay and Ford (1947). For the experiments in this paper the Hilger (D 33) monochromator was used almost exclusively.

The radiant energy incident on the sensitive spot is, of course, different from the energy incident on the spore as measured by the thermopile. It is evidently of importance to obtain as much informa-

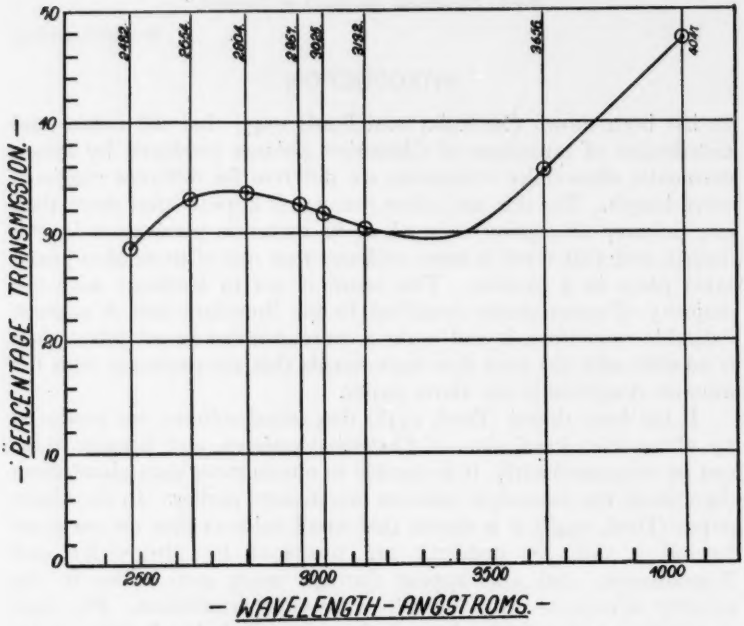


FIG. 1.—Percentage of the incident energy transmitted through half the spore given by experiments using a layer of spores.

tion as possible about the absorption in the spore. This is important, not only because of the absolute magnitudes involved, but because the shape of the reciprocal dose-wave length curve may depend on this quantity.

This was pointed out in the paper (McAulay and Ford, 1947) where it was shown that in an extreme case the reciprocal dose-wave length curve characteristic of nucleic acid might appear from the thermopile measurements, uncorrected for absorption, to resemble a curve characteristic of protein absorption.

Two methods were used to measure ultra-violet absorption in the spores. In both the absorption was found by comparing the relative

times required for a given film blackening, in one case through spores, in the other case by direct irradiation. The blackening was determined photoelectrically, using a microscope to enlarge a small area of film to a suitable working size.

As no quartz microscope lenses were available in the laboratory, the method at first used had to depend on absorption in a layer of spores. These were spread behind a pinhole 250 μ in diameter. Results obtained in this way showed an absorption curve giving a transmission band in the neighbourhood of 2804 A.U. There was not sufficient difference, however, between the radiation transmitted at 2804 A.U. and at neighbouring wave lengths to affect greatly the form of the curve which would be obtained if direct thermopile readings had been taken as giving energy incident on the sensitive spot.

Some months ago Mr Matthei of the Science Faculty Workshops, University of Melbourne, kindly lent us a set of quartz microscope optics and it has been possible to make absorption measurements directly on the image of a spore.

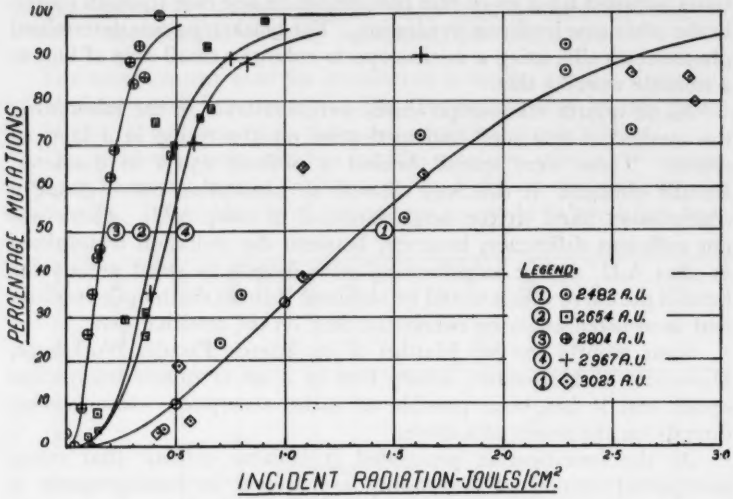
As the investigation proceeded it became evident that many unexpected sources of uncertainty are present in measurements at high magnification of absorption in the material of the spore, and a reliable result from experiments with single spores is not in sight. However, measurements on single spores confirm generally the results obtained with a layer of spores. Fig. 1 shows the percentage of the incident energy transmitted through half the spore given by experiments using a layer of spores.

It should be noted that even if completely satisfactory absorption measurements can be made, the energy incident on the sensitive spot is still in doubt as the distribution of the absorbing material in the spore and the position of the sensitive spot are both involved, and in present conditions determination of these points is not in sight.

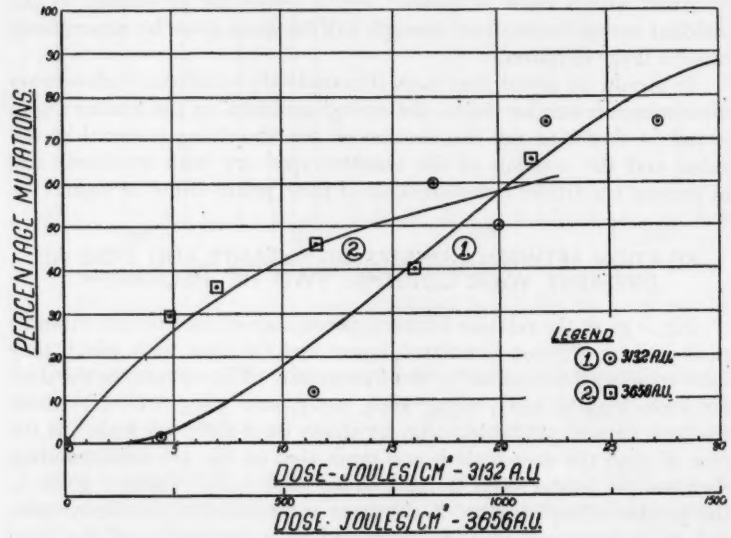
RELATION BETWEEN NUMBERS OF MUTANTS AND DOSE AT DIFFERENT WAVE LENGTHS. TWO HIT MECHANISM

Fig. 2 gives the relation between percentage of microscopic mutants produced by growing irradiated spores and the dose with which they were irradiated measured by the thermopile. Fig. 2*a* records the data for wave lengths 2482, 2654, 2804, 2967, and 3025 A.U. for which the best data is available. Fig. 2*b* shows on a different scale (in the case of 3656 the dose scale is 500 times that on fig. 2*a*) corresponding data for the longer wave lengths 3132, 3656 A.U. Table 1 gives A, the number of experiments, B, the doses as measured by the thermopile, and C, microscopic mutants expressed as a percentage of the total number of germinating spores. This data is given for wave lengths from 2482 A.U. to 3656 A.U. The total number of mutants examined to provide data for this table amounts to 1381 colonies.

A fuller investigation of the relation between microscopic mutants



(*a*) Records data for wave lengths 2482, 2654, 2804, 2967, 3025 A.U.



(*b*) Shows on a different scale (in the case of 3656 the dose scale is 500 times that of Fig. 2*a*) corresponding data for the longer wave lengths 3132 and 3656 A.U.

FIG. 2.—Percentage of microscopic mutations obtained with different ultra-violet wave lengths plotted against incident dose in joules/cm.².

TABLE 1

Showing the relation between dose and percentage microscopic mutants obtained in sampling experiments at different ultra-violet wave lengths

A Number experiment	B Dose in joules/cm.²	C Percentage microscopic mutants
2482 A.U.		
1	0·5	10
	1·0	33
	2·6	73
2	0·0	3
	0·702	24
	1·54	53
	2·29	87
3	0·44	4
	0·80	35
	1·62	77
	2·3	93
	...	...
	...	...
2804 A.U.		
1	0·138	44·0
	0·225	69·0
	0·332	92·3
	0·428	100·0
2	0·018	3·0
	0·0366	0·0
	0·0549	3·0
	0·0915	27·0
	0·1647	47·0
	0·2975	89·0
3	0·1062	37·1
	0·2075	62·2
	0·3071	84·6
	0·3718	86·4
4	0·0158	0·7
	0·049	9·0
3025 A.U.		
1	0·0	0·0
	0·57	6·0
	1·77	74·0
	2·88	80·0
2	0·0	4·0
	0·52	19·0
	1·09	40·0
	1·64	63·0
	2·6	87·0
3	0·106	0·0
	0·421	4·3
	1·093	66·0
	2·853	85·0
	...	...
	...	...
	...	...
	...	...
	...	...

A Number experiment	B Dose in joules/cm.²	C Percentage microscopic mutants
2654 A.U.		
1	0·14	7·5
	0·268	29·4
	0·37	30·4
	0·49	70·0
	0·67	78·3
2	0·1	2·5
	0·24	3·2
	0·36	25·8
	0·483	68·2
	0·622	77·3
3	0·417	75·0
	0·637	92·6
	0·904	98·6
2967 A.U.		
1	0·0	0·0
	0·1	0·0
	0·58	71·0
	0·82	89·0
	1·62	90·5
2	0·0	0·0
	0·39	36·0
	0·75	90·0
	...	...
	...	...
	...	...
	...	...
	...	...
	...	...
	...	...
3132 A.U.		
1	0·0	0·0
	20·0	50·0
	38·0	98·0
2	4·35	2·0
	11·41	2·0
	16·94	60·0
	22·94	74·0
	27·33	74·0
3656 A.U.		
1	240	29·0
	350	37·0
	577	46·0
	806	40·0
	1078	66·0

and dose was made at the wave length 2804 A.U. than at other wave lengths. The object was to obtain evidence about the number of hits on a sensitive spot required to produce the microscopic mutants. Fig. 3 shows the curve obtained experimentally compared with

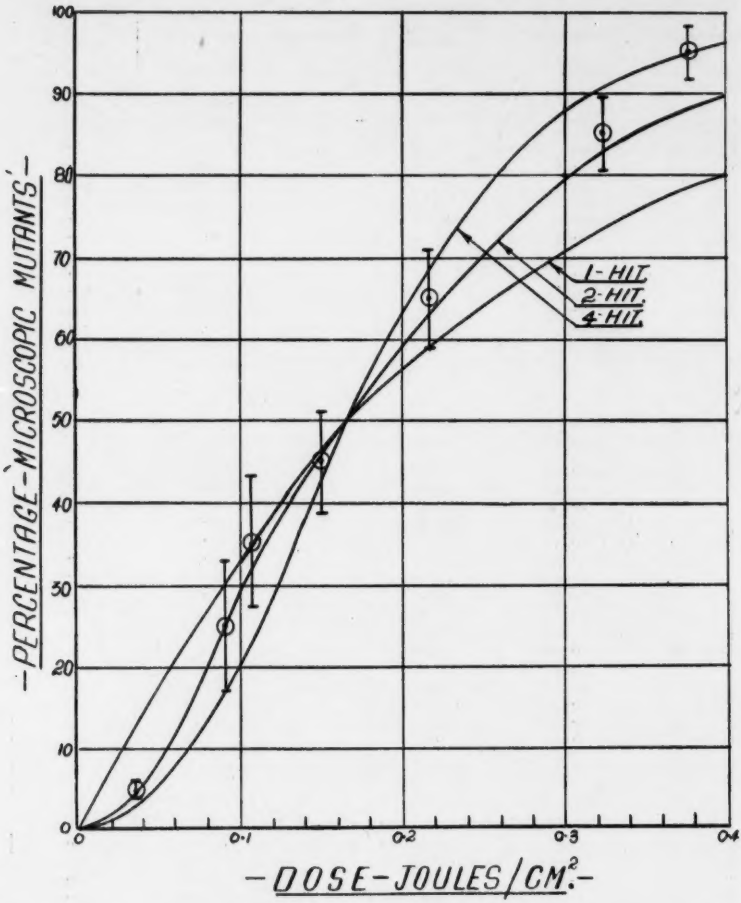


FIG. 3.—The relation between microscopic mutants and dose at the wave length 2804 A.U., with the standard deviation of each point shown as a vertical line drawn either side of the point. Theorectical curves for 1, 2 and 4 quantum hits are drawn on an appropriate scale.

theoretical curves for one, two and four hits, drawn on an appropriate scale. The results are exhibited in this curve in a different way from those of fig. 2, the object being to show as clearly as possible the significance of its shape. Results are collected at each 5 per cent.,

the doses required to produce between, say 20 per cent. and 30 per cent. mutations being averaged and plotted against 25 per cent. mutation. This treatment enables the standard deviation to be plotted. The standard deviation at each point is shown in the curve by the vertical line. The critical point at 5 per cent. mutations has a very small standard deviation as 400 colonies were grown at small dosage because of the importance of this part of the curve. Table 2

TABLE 2

Giving standard deviation of percentages of microscopic mutants $(pq/n)^{\frac{1}{2}}$ *obtained in experiments at 2804 A.U.*

p per cent.	n	$(pq/n)^{\frac{1}{2}}$	Average dose in joules/cm.^{2}
5	401	1·09	·035
15	...	...	...
25	30	7·91	·091
35	35	8·07	·106
45	61	6·38	·151
55	...	...	...
65	66	5·88	·216
75	...	...	...
85	66	4·40	·326
95	42	3·37	·380

lists results exhibited in fig. 3, and shows, p, the percentage of microscope mutants, n, the number of spores from which the mutants were obtained, $(pq/n)^{\frac{1}{2}}$ the standard deviation, in which q is $100-p$, and in the last column the average dose required to produce the mutants. It is seen that the experimental results are consistent with a two hit mechanism. The effect of variable depth of the absorbing sensitive spot would be to make the number of hits appear smaller than is actually the case.

It is not considered that the data for other wave lengths justifies any definite conclusion, but it may be noted that except for the case of 3656 A.U. which suggests a single hit mechanism the curves are consistent with a two hit mechanism.

EFFECT OF INTENSITY

Some experiments were made at the ultra-violet wave length 2804 A.U. to test the effect of dose-rate. It was noted that over a 10 : 1 range of intensity about the region normally used for irradiation no noticeable change in effect occurred. A few experiments were made over a 200 : 1 range of intensity, and the change in effectiveness was less than 2 : 1 over this dose-rate. The indication is that very small intensities and very high intensities are more effective than the mid-range, but points are too few and too scattered to say anything definite here.

RECIPROCAL DOSE-WAVE LENGTH RELATION FOR 50 PER CENT. MUTANT PRODUCTION

It will be seen that the radiation is most effective at 2804 A.U. In fig. 4, curve A, the reciprocal of the dose required to produce 50 per cent. microscopic mutants taken from fig. 2 is plotted against

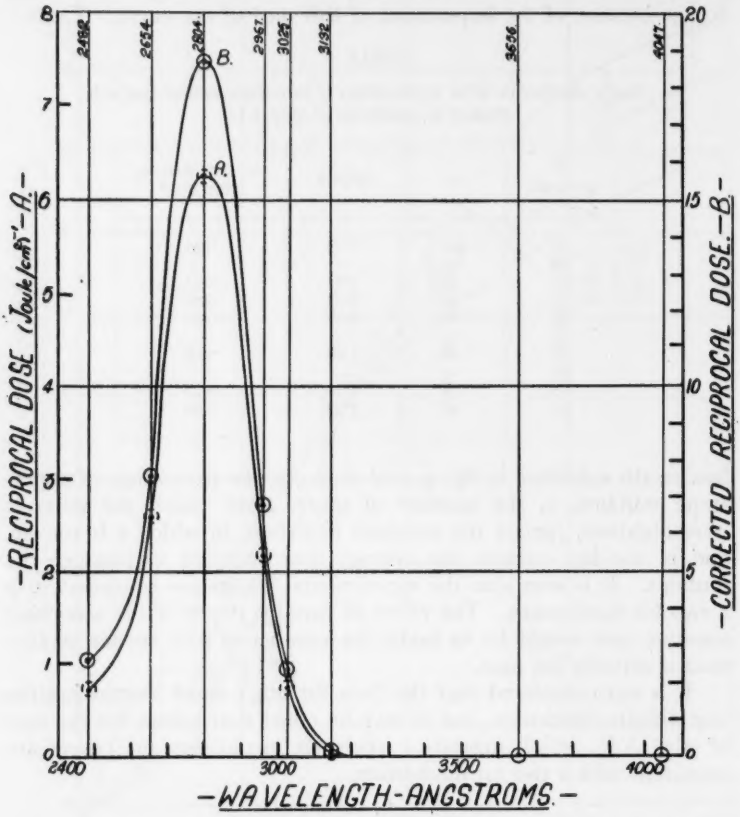


FIG. 4.—The reciprocal dose required to produce 50 per cent. microscopic mutants taken from Fig. 2, plotted against wave length.

(*a*) Uncorrected for absorption in the material for the spore.
(*b*) Corrected for absorption in the material of the spore.

wave length. Curve B gives the reciprocal dose corrected for absorption in the material of the spore, and is intended to give rough figures for the reciprocal dose at the sensitive spot, absorption in which gives rise to mutations. It must be realised that the data for the corrections which give curve B from curve A is extremely suspect. It assumes that the sensitive spot is in the middle of the spore, and

makes use of absorption measurements, made on a layer of spores. Absorption measurements made with single spores have not yet been sufficiently self-consistant for use. The reciprocal dose is used because the amount of radiation absorbed by a small thickness dx is given by $dI = -\mu I dx$. Assuming that the same amount of energy absorbed

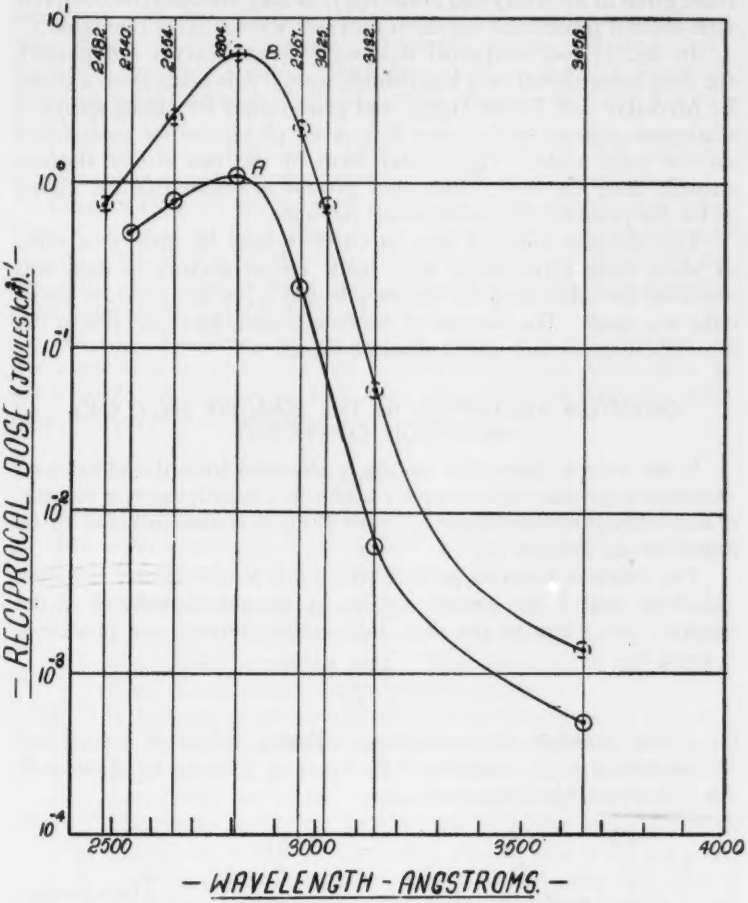


FIG. 5.—Reciprocal dose-wave length curves, dose being shown on a logarithmic scale.

(*a*) Gives results for killing spores of *Chætomium globosum*.

(*b*) Shows curve 4 (*a*) plotted for a comparison on the same scale.

produces the same effect at different wave lengths, the reciprocal dose $1/I$ will be proportional to the absorption coefficient μ of the sensitive region.

It will be seen that the result is similar to that obtained with

macroscopic mutants (McAulay and Ford, 1947) and that, as pointed out in the earlier paper, the curve with these general characteristics is much more consistent with a process of absorption in a protein than in nucleic acid.

The results in the present paper are on a firmer foundation than those given in McAulay and Ford (1947) as they are based on complete dose-mutant production curves at different wave lengths (see table 1).

In fig. 5, two reciprocal dose-wave length curves are plotted, the dose being shown on a logarithmic scale ; A is taken from a paper by McAulay and Taylor (1939) and gives results for killing spores of *Chætomium globosum* while curve B is curve 4A plotted for comparison on the same scale. The similar form of the two curves suggests strongly that the same absorption process is responsible for killing as for the production of microscopic mutants.

The absolute value of dose in curve A may be greatly in error as when these experiments were made no satisfactory method was available for calibrating the thermopile and a low temperature Leslie cube was used. The interest of the comparison, however, lies in the form of the curve and not in absolute values.

QUANTUM ABSORPTION IN THE SENSITIVE SPOT AND ABSORPTION COEFFICIENT

If the view is taken that energy is absorbed from the ultra-violet radiation to produce microscopic mutants in a sensitive spot of volume, v, and absorption coefficient μ, curve B fig. 4 enables a value to be found for the product μv.

The relation between μ, v, λ (the wave length of the radiation absorbed) and I the energy density in the neighbourhood of the sensitive spot when 50 per cent. microscopic mutants are produced, is given by

$$\mu v = \frac{1 \cdot 65\ hc}{I\lambda}$$

for a two quantum hit mechanism. Taking values of I corrected for absorption in the material of the spore as given in fig. 4 curve B, the following table is obtained :—

I λ	II $v\mu$ Product of volume of sensitive spot and absorption coefficient	III μ Absorption coefficient of sensitive spot. Assuming $v = 3 \cdot 3 \times 10^{-19}$ cc.	IV μ For 2 per cent. nucleic acid
2482	$34 \cdot 0 \times 10^{-21}$	·102	·74
2654	$91 \cdot 8 \times 10^{-21}$	·276	1·11
2804	$215 \cdot 1 \times 10^{-21}$	·645	·46
2967	$74 \cdot 3 \times 10^{-21}$	·222	·14
3025	$25 \cdot 0 \times 10^{-21}$	·075	...
3132	$1 \cdot 86 \times 10^{-21}$	·0057	...
3656	$0 \cdot 036 \times 10^{-21}$	·000108	...

The table gives in column II the product $v\mu$ derived from data such as that given in fig. 4, curve B the corresponding wave lengths in Angstroms being given in column I. In column III the value of μ is tabulated on the assumption that the volume of the sensitive spot is $3{\cdot}3 \times 10^{-19}$ cc.

This value for the volume of the sensitive spot is chosen as reasonably consistent with that obtained by a number of investigators using a wide range of living material, *Drosophila* sperm (Gowen, 1936), Bacteriophage (Lea, 1946), White Eye mutation gene in *Drosophila* (Haskins and Enzmann, 1936), *Euglena* (Swann and del Rosario, 1932).

It is also the volume of a large protein molecule and anything much smaller would appear to demand a different type of theory.

Column IV gives the value of μ for 2 per cent. nucleic acid taken from a curve given by Caspersson in a symposium on nucleic acid (1947). It will be seen that our values of the absorption coefficient are of the same order of magnitude, although of course their variation with wave length is quite different.

The data for absorption in protein seems very variable and as the absorption in which we are concerned probably takes place in some combined nucleo-protein it does not appear particularly useful to give figures for comparison.

SUMMARY

The main results from an extensive investigation of the production by ultra-violet irradiation of microscopic mutants of *Chætomium globosum* involving the growth of about 1400 mutants, are as follows :—

(i) The reciprocal dose-wave length curve is quite unlike that to be expected if the mutations were produced by a primary absorption in nucleic acid. The curve is of the general form characteristic of absorption in proteins.

(ii) The product of the volume of an absorbing sensitive spot and its absorption coefficient can be obtained from our results. If a value of 3×10^{-19} cc. is assumed for the volume in accordance with the results of most experimenters, the absorption coefficient in our case is of the same order of magnitude as that of 2 per cent. nucleic acid or 60 per cent. protein. These figures are only generally suggestive as it is probable that the absorption actually takes place in some nucleo-protein.

(iii) The curve connecting dose and mutant production is consistent with absorption by a two hit mechanism. Careful experiments were made with a wave length of 2804 A.U. particular attention being paid to the low dose part of the curve to settle with some degree of certainty that more than one hit was involved.

(iv) There is no marked effect of radiation intensity on the effectiveness of production of mutants. Experiments to test this point were made solely with the 2804 A.U. line.

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BRITISH ASSOCIATION, 1948

SYMPOSIA ON GENETICS

SECTIONS D, H AND I

HUMAN BLOOD-GROUPS

ABSTRACTS of Papers read on MONDAY, 13th SEPTEMBER

BLOOD-GROUPS AND HUMAN GENETICS

J. A. FRASER ROBERTS

Apart from the blood-groups there are exceedingly few common, simply inherited human traits. Thanks to the fact that dominance is often absent, or at least incomplete, the elucidation of the genetics of the blood-groups has followed closely on advances in serology ; while their great practical importance has led to the testing of millions of donors, thus permitting applications to geographical and racial variations without parallel in other living forms. A knowledge of the simple elements of genetics, together with an appreciation of the importance of multiple alleles and of Fisher's hypothesis of closely linked (or compound) genes, makes it a relatively simple matter for those who are not themselves serologists to follow these important recent advances in genetics and anthropology.

THE MULTIPLICITY OF BLOOD-GROUPS IN MAN

R. R. RACE

Blood-groups are not yet as individual as finger prints, but their inheritance is known much more precisely. There are seven firmly established systems of groups, called ABO, MNS, P, Rh, Lutheran, Lewis and Kell. The groups are determined by antigens on the red blood cells ; and an antigen is recognised when the cells carrying it are clumped, or agglutinated, by a serum containing the corresponding antibody.

The presence of an antigen is under very rigid genetic control. As far as is known the genes for each of the groups are carried on separate chromosomes, though loose linkage may yet be demonstrated between some of them.

In England the seven groups make over 20,000 recognisably different combinations ; these vary greatly in frequency. The commonest combination has a frequency of 2 per cent., while the calculated frequency of the rarest is so low that it may never have formed the blood of an Englishman.

BLOOD AND SPEECH

C. D. DARLINGTON

It has long been obvious that the enduring processes of differentiation in the use of sounds by spoken languages depended on the genetic differences of the races speaking them. Critical evidence however had to wait for the gene mapping of

large populations. This became possible with the development of blood-group surveys. A contour map of Europe for the O blood-group shows a significant relation with the history of human migrations during the last 3000 years. It also shows a significant agreement with the present distribution of the TH sound in European languages and its historic changes. It is now therefore possible to undertake the study of the relations of race and language on rigorous genetic foundations with consequences equally remarkable for the study of evolution in man and in the language he speaks.

THE ETHNOLOGICAL DISTRIBUTION OF THE Rh AND MN BLOOD-GROUPS

A. E. MOURANT

Whereas the frequencies of the ABO blood-groups show significant variations over distances of the order of 100 km., the MN and Rh groups, though less is known of their distribution, appear to vary much more gradually.

The MN groups show an almost constant frequency all over the world with certain exceptions. M is more than normally frequent among the Asiatic Indians, and among the North American Indians and Eskimos and, to a less marked extent, among the Baltic peoples. N is abnormally frequent among the Ainu of Japan, the Papuans and the Australians. MN distributions have, up to now, thrown little light on racial relationships. The Rh system gives promise of throwing much light on such connections.

In Asiatics, American Indians and peoples of the Pacific the CDe (R_1) and cDE (R_2) gene combinations predominate in varying proportions. Among Negroes cDe (R_0) is by far the commonest combination and shows that the Negroes are quite different from all the so-called Negroids of the east so far tested.

In Europe CDe and cDE are common, together with cde (r). Rh— negative people (cde/cde) amount to about 16 per cent. throughout most of Europe. Since there is a selective elimination of heterozygous Dd babies of dd mothers as a result of hæmolytic disease of the newborn, the present Rh frequencies in Europe appear to be unstable if considered over a long period. It is probable that the present distribution is due to a mixing, at the end of the palæolithic period, of a stock akin to the modern Basques, who have nearly 30 per cent. of dd (mostly cde/cde) individuals, with a predominantly Rh positive (or D) stock from the east.

HÆMOLYTIC DISEASE OF THE NEWBORN

P. L. MOLLISON

Hæmolytic disease of the newborn is a comparatively common condition, affecting about 0·5 per cent. of all infants born in this country. The very special interest of this disease lies in the fact that it has been shown to be due to a particular blood group difference between the parents. In brief, the mechanism is this : the father hands on to the fœtus an antigen, Rh, which is foreign to the mother ; the mother forms antibodies against this antigen and these enter the fœtal circulation during pregnancy and cause destruction of the fœtal erythrocytes. If the infant is born alive, the hæmolytic process comes to an end as the infant is cut off from the supply of destructive antibodies. The disease in man is paralleled by a similar condition in newborn mules. In this case the mule inherits from its sire, the donkey, an antigen which is foreign to the mare. The mare forms antibodies which in turn cause destruction of the mule's erythrocytes.

The serological basis of the disease in man has only been understood since 1941. This knowledge has proved invaluable in understanding the disease and managing it.

For instance, the occurrence of the disease can be predicted by routine testing of pregnant women for the presence of Rh antibodies and this has, in fact, become a standard practise in many hospitals. Again, Rh negative blood can be selected for the transfusion of the infant with results greatly superior to those of transfusing unselected blood.

These two pieces of knowledge are combined in the most recent treatment of this condition. Labour is induced prematurely to diminish the period for which the fœtus is exposed to Rh antibodies and a massive exchange transfusion is then carried out, some 90 per cent. of the infant's own blood being progressively removed and replaced by Rh negative blood.

SECTIONS M, D AND K

INBREEDING AND HYBRID VIGOUR IN LIVESTOCK AND CROP IMPROVEMENT

ABSTRACTS of Papers read on FRIDAY, 10th SEPTEMBER

THEORY OF INBREEDING

R. A. FISHER
Cambridge

From the point of view of pure genetics inbreeding should be regarded as a means of manipulating the germ plasm so as to control its structure and composition, particularly in respect to the numerous invisible factors which cannot be recognised individually. In practical plant improvement this tool has proved to be enormously successful, and its success makes it certain that it will be extensively used both in animal and plant improvement in the future. A theoretical understanding of its *modus operandi* seems, however, to be lacking, and even in the field of maize genetics, where the greatest amount of work has been done, very diverse opinions are held as to the reason for its success.

Elementary considerations show that the manifest effect of inbreeding must be due to dominance, and the genetical theory of the origin of dominance throws some light on the phenomena observed in inbreeding. Selection is exercised in three distinguishable phases of the process of producing desirable hybrids. The relative importance of selection at these stages is, however, obscure and disputed. It is submitted on quantitative grounds that the essential advantage of an inbreeding programme consists in the production in great variety of material which is reliable in its breeding properties, and that it is the facility offered for increasingly accurate selection by this reliability that constitutes the essential advantage conferred by inbreeding. In the work of maize improvement a very important contribution to the accuracy of selection at this stage has been the rapid improvement in the accuracy of varietal trials supplied by replicated randomised experimentation.

HYBRID CORN (MAIZE) IN THEORY AND PRACTICE

G. M. L. HASKELL
Bayfordbury

Corn (*Zea mays* L.) is grown in many parts of the world for animal and human consumption. Between 1930 and 1947 there has been a change over from corn varieties to hybrids in the mid-western States of America.

The species is naturally cross-pollinated by wind and genetically plants in a variety are heterozygous. Ordinary selection methods for improving varieties, *e.g.* increasing yields, do not lead to improvements. Other breeding techniques are therefore necessary.

Inbreeding corn produces a great variety of offspring. Continued selection and inbreeding produces inbred lines showing inbreeding depression but reasonably uniform and having satisfactory plant characters. Inbred strains hybridised together produce offspring showing increased vigour, such hybrids often surpassing both parental varieties in yielding capacity. Each plant is a potential yielder and the hybrid generation is uniform in appearance and in maturity.

Greater hybrid vigour results using inbreds of diverse parentage and different inbreds have different abilities to combine well with others. This is a genetical property which can be bred for.

The theories of hybrid vigour are outlined.

The various methods of inbreeding and hybridisation in commercial use are described for sweet corn (crossing two inbreds) and field corn (crossing two different F_1's). Methods recently introduced here are given and the future of hybrid corn in this country and in the Commonwealth discussed. The co-operation of the seed trade is stressed.

CROSSBREEDING AND INBREEDING IN LIVESTOCK PRODUCTION

H. P. DONALD

Edinburgh

There are four ways of attempting to get better performance by breeding, and the manipulation of breeding systems is one of them. It is convenient to regard inbreeding and crossbreeding as opposite deviations from outbreeding. This has the drawback, however, that outbreeding has not, in practice, the precise meaning of random matings in a closed population, but can, and does, include at times considerable elements of both types of deviation from it.

Crossbreeding has several distinct functions arising out of its genetical consequences which are (*a*) the production of a comparatively uniform and intermediate F_1; (*b*) a subsequent increase of genetic variation; and (*c*) heterosis.

Examples of (*a*) and (*b*) are :—The stratification of sheep; the importation of Canadian Tamworths and Friesians; the use of the zebu in beef cattle country of Queensland; the use of Cheviot crosses in deteriorating New Zealand pastures; the creation of new breeds. Theoretically, heterosis should enter into these examples, and probably it does, but it is difficult to prove.

Inbreeding leads to (i) an increase of homozygosity; and (ii) the canalisation of genetic variability into differences between lines instead of among individuals. Inbreeding, even at modest rates, outstrips selection and livestock deteriorate. Subsequent crossing of lines may recover lost vigour. Occasionally, the performance of foundation animals may be exceeded.

Considerations of time and numbers make inbreeding an unsuitable technique for most pedigree breeders. The trend is to leave the risks and rewards to institutions, *e.g.* the Regional Swine Breeding Laboratory and the Hy-line chicks in the U.S.A. Organised crossbreeding works because it fulfils well-understood functions and is carried out on a breed and area basis. Inbreeding as yet has only vague intentions and depends too much on the individual animal, herd and man. The more a breeder deviates towards inbreeding, the more likely he is to end by deviating towards crossbreeding.

INBREEDING AND HYBRID VIGOUR IN THE IMPROVEMENT OF SWEDISH SUGAR BEET

J. RASMUSSON
Hilleshög

The effects of inbreeding in sugar beet are: genical homogenisation (showing especially in leaf morphology) and depression of productivity and adaptability to varying external conditions, whereas chemical constitution of the root (content of sugar, K+Na, N) is not perceptibly influenced by inbreeding in itself. Size characters do not show decrease in phenotypical variation, probably because their lower vitality makes them react more to the variation in external conditions. For producing types with specific characters to which yield and vitality are not important, inbreeding is the most effective means. Inbreeding may also be of use in breaking up positive correlations between desirable and undesirable characters caused by linkage.

Selfing in the author's material has not given rise to any practically useful types. Breeding by repeated selection of mother-plant families has produced some practically valuable families. Rigorous mass-selection under specified growing conditions shows some of the traits of inbreeding, *i.e.* a marked degree of genical homogenisation and stability together with decrease in adaptability. If rigorous mass selection is combined with reproduction from few parent plants even a decrease in vitality and yield may occur.

Crossing genically different types nearly always gives an increase in yield and adaptability above the parental average, and hybrids between parents not too much differing in yield mostly surpass the better parent. The use of parents of genealogically different origin usually further improves yield but this effect may simply be the result of the difference in origin resulting in greater difference between the gene complements.

Hybridisation seems to have a very slight effect upon content of sugar (decreasing), K+Na (increasing) and N (decreasing) as compared with the parental average. Hybrids between family selected strains usually do not produce as high a yield as hybrids between comparable strains genically differentiated by mass-selection under different growing conditions.

The triploids may be considered as a special case of heterozygosity. They surpass the diploids by 4-5 per cent. in yield, and the diploids in their turn surpass the tetraploids by 5-10 per cent.

GENETICAL CONSIDERATIONS IN THE UTILISATION OF HYBRID VIGOUR

K. MATHER
Birmingham

The method of inbreeding and subsequent crossing of the inbred lines is used in naturally crossbreeding species, where homozygous lines, though of course uniform, are uniformly poor. The aim of the method is to produce heterozygotes uniformly approaching the best individuals possible in open-bred varieties, and so, as groups, exceeding those varieties; and to produce such desirable heterozygotes predictably, generation after generation.

Five problems are raised by this method of breeding :—

1. A great multiplicity of inbred lines can be raised. These must all be tested and rapid methods of sorting them out are needed. The value of an inbred line in crosses is not predictable from its own production. Combining ability is the critical character which must be measured and made the object of selection.

2. The inbreds are themselves very poor, expensive to keep and give low yields of hybrid offspring in immediate crosses. Double crossing, top crossing, and convergent improvement are all methods aiming in various ways at overcoming this difficulty.

3. The deliberate raising of hybrids demands means of making controlled crosses on a large scale. In animals and maize this is easy; but plants with hermaphrodite flowers demand special methods. Bulk crossing in sugar beet, and the use of cytoplasmic male sterility in onions have been developed for this purpose.

4. The very uniformity of the inbreds and hybrids carries with it certain dangers. That of the inbreds leads to risk of loss of valuable genetic variation, such as is automatically maintained in the reservoir of variability of the open-bred varieties. Collections of these varieties may therefore be necessary to provide sources of future breeding material.

In the hybrids, uniformity leads to the risk of serious loss of crops through the temporary supervention of adverse conditions whose effects would be less serious on a more variable stand. Double crossing goes some way to overcome this risk.

5. The method of inbreeding and crossbreeding must inevitably be more costly than more conventional techniques of breeding. There is thus the ever present question of whether the method will pay for itself by the gains to which it leads. And we can have no certainty that the answer will be the same for all species in all circumstances.

SECTION K

THE GENETICAL STRUCTURE OF PLANT POPULATIONS

ABSTRACTS of Papers read on MONDAY, 13th SEPTEMBER

THE GENETICAL STRUCTURE OF PLANT POPULATIONS IN RELATION TO GEOGRAPHICAL AND ECOLOGICAL DISTRIBUTIONS

W. B. TURRILL

Kew

The extensive and intensive studies now being completed on numerous populations of a limited number of species of the European flora have revealed an unexpected wealth of variation in structure and behaviour. Some of the variations are common, others are rare; some have obvious positive and general survival value, some are more or less lethal or harmful, some appear to be neutral from the standpoint of survival within any population; some are correlated with local environments, some have wider geographical ranges. Hybridisation experiments have shown that most of the variations found in the wild are due to genetic differences and that the operation is often by the interaction of a larger or smaller number of determinable genes. Some, however, are not yet fully understood genetically.

Genetical experiments must be combined with field and herbarium studies if the history of the plants is to be satisfactorily elucidated. The value of the information given by cytology varies with the group.

The above generalisations will be drawn from the consideration of a few groups of species which have been studied by combined methods.

INTRAPOPULATION HETEROGENEITY AND THE BREEDING RANGE OF THE INDIVIDUAL PLANT

J. L. CROSBY
Durham

In addition to reciprocally outcrossing pins and thrums, certain populations of *Primula vulgaris* contain long homostyles which are normally self-fertilised and outcross on to pins. Observation agrees with the theoretical conclusions that homostyles are increasing to about 80 per cent., pins are decreasing to about 20 per cent., and thrums are entirely disappearing. Slow at first, change later becomes quite rapid.

Considerable heterogeneity between different parts of any one such population may exist. A few yards only may separate groups with constitutions so different that they represent evolutionary stages many generations apart. Such large differences cannot have arisen in one generation by chance from a homogeneous population, but must have been so initiated at an early stage. One section of the population reached the stage of more rapid change, the difference then becoming amplified. But it could not even be maintained with free distribution of pollen between the divergent groups, even though homostyles are self-pollinated.

The breeding range of the individual *P. vulgaris* must therefore be only of the order of a few yards, and a single population cannot be considered as a continuous interbreeding unit. Discussion of the significance of this for general evolution theory must take account of the abnormal rapidity of homostyle evolution.

POPULATIONS OF CULTIVATED PLANTS

A. J. BATEMAN
Bayfordbury

Populations of cultivated plants are simpler in several respects than natural populations. Their study can therefore help to illuminate the even more complex situation in wild plants.

The main factor determining population structure is the breeding system. Subordinated to this are selection intensity, population size and gene dispersal.

The breeding system determines the free and potential heritable variation and the rate of liberation of potential variation. Cultivated plants show a continuous range in breeding systems from self-imcompatibility and dioecism giving full outbreeding, to regular self-pollination and vegetative reproduction, giving full inbreeding or its equivalent.

Cultivated plants are selected for a special environment. When human selection is relaxed the subsequent deterioration shows us how natural populations with similar breeding systems will respond to changes of environment.

Population size affects the maximum heritable variation which can be carried and, when very small, the amount of inbreeding in otherwise outbreeding species.

A study of the dispersal of pollen of cultivated plants shows that intercrossing is mainly confined to immediate neighbours, though finite amounts of crossing occur up to large distances. The part which this dispersal plays in determining population structure again depends on the breeding system.

GENE-FLOW BETWEEN INTER-FERTILE SPECIES

H. G. BAKER
Leeds

Attention is drawn to Allan's distinction between *multiform* and *pauciform* natural hybrids. In some cases, natural gene-flow between forms shown to be inter-fertile when crossed artificially appears to be restricted. Recently described cases of this sort are examined. The possible causes of restriction are enumerated and discussed. The stages which may occur in the invasion of a region containing one form by another, closely related and inter-fertile form are outlined and illustrated. An important distinction is drawn between the flow of genes controlling characters of significance in the ecological difference between the forms and those which are neutral in this respect.

INTERSPECIFIC HYBRIDISATION IN *PRIMULA*

D. H. VALENTINE
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Primrose (*Primula vulgaris*) and Oxlip (*P. elatior*), in certain regions of East Anglia, occur only in woodland ; the populations of individual woods are thus isolated from one another. Some woods contain one or other of the pure species, others contain the two species and their hybrids.

Where the species meet, they occupy distinct areas in the wood, probably because of their different ecological preferences. Hybrids are always frequent but they do not outnumber the pure species. From reciprocal artificial crosses, a low yield of viable seeds is obtained, and the vigour of the F_1 hybrids is variable. A somewhat better yield of seed is obtained when the F_1 hybrid is selfed or backcrossed.

There are thus both ecological and genetical barriers which tend to present fusion of the species. It has been suggested that Primrose is replacing Oxlip in the mixed populations, but the available evidence suggests that the condition is one of equilibrium, which may in the course of time shift in one direction or the other as variations of local climate and environment occur.

Efficient barriers of more than one kind separate Oxlip and Cowslip (*P. veris*), and the hybrid between them is very rare. The hybrid between Primrose and Cowslip, which is partially fertile, is of widespread occurrence, but extensive hybrid populations are apparently rare, perhaps because of the marked ecological differences between the species.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the NINETY-EIGHTH MEETING of the Society, held on WEDNESDAY, 15th DECEMBER 1948, in the Rooms of the LINNEAN SOCIETY, Burlington House, Piccadilly, London, W. 1

NEW OBSERVATIONS ON THE INFECTIVE SPREAD OF NATURAL SKIN PIGMENTATION IN RODENTS

R. E. BILLINGHAM and P. B. MEDAWAR
Department of Zoology, The University, Birmingham

Relation between red, black and white phenotypes; propagation of infection from animal to animal; cure of the infection.

MYCETOZOA AND GENETICAL RESEARCH

E. A. BEVAN
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In certain Orders of Mycetozoa a plasmodial stage (*i.e.* multinucleate structure showing rythmical cytoplasmic flow) precedes the formation of a characteristic fruiting body, the spores of which give origin to flagellate or amœboid cells. The plasmodium is believed to originate from the fusion of two or more such cells. In other Orders the plasmodium is not formed, but numerous amœbæ come together to form a structure (pseudoplasmodium) in which they remain as distinct uninucleate cells not further dividing; this colonial structure as such forms the fruiting body.

Joint cytological and genetical work is required for the understanding of the nuclear cycles, and in particular at which stages in the life-cycle karyogamy and meiosis take place, and of the genetic system. The Class offers the possibility of studying in the same species the physiological genetics of uninucleate amœbæ, multinucleate plasmodia (with the possibility of heterokaryosis) and differentiating structures (fruiting bodies). In different species a plasmodium can be compared with a pseudoplasmodium, and the morphogenetic process in the two situations can be analysed.

The genetical approach requires "markers" visible or nutritional. The difficulties include the present inability to keep any mycetozoon on a non-living diet and the great amount of non-inheritable variation. Work is in progress with *Didymium nigripes*.

THE BREAKDOWN OF CHIASMA INTERFERENCE IN INTERCHANGE ASSOCIATIONS

H. G. CALLAN
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The number of chiasmata formed between the bivalents of *Forficula auricularia* is limited by interference across the centromere. Arm interchange results in the formation of multivalent associations whose chiasmata are not so limited, proving that chiasma interference is a relational property shared by pairing partners.

A STUDY OF ADJACENT GENES

E. B. LEWIS
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The mutants bithorax (*bx*) and bithoraxoid (*bxd*) in chromosome 3 of *Drosophila melanogaster* transform the metathorax towards a mesothoracic-like structure, notably producing wing-like halteres. The *bx* mutants are discovered to transform only the anterior portion of the metathorax in this manner, while *bxd* and *bxd*/*bxd*^{D} transform the posterior part correspondingly. The *bxd* mutants also modify the first abdominal segment towards a metathoracic-like one, notably producing first abdominal, metathoracic-like legs. Genetic studies show that *bxd* and *bxd*^{D} lie just to the right of *bx*, and that a position effect exists between *bx* and *bxd*^{D}. On cytological grounds *bx* and *bxd*^{D} are assumed to be repeated genes. It is presumed that one of these genes diverged from the other in evolution by governing a reaction successive to that of the original gene and that the position effect relates to the dependence of one gene on the reaction product of the other.

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